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ABSTRACT

A method was developed for the direct determination of mercury in water and biological samples using a unique carbon bed atomizer for atomic absorption spectroscopy. The method avoided sources of error such as loss of volatile mercury during sample digestion and contamination of samples through added reagents by eliminating sample pretreatment steps.

The design of the atomizer allowed use of the 184.9 nm mercury resonance line in the vacuum ultraviolet region, which increased sensitivity over the commonly used spin-forbidden 253.7 nm line.

The carbon bed atomizer method was applied to a study of mercury concentrations in water, hair, sweat, urine, blood, breath and saliva samples from a non-occupationally exposed population. Liquid samples were introduced into the atomizer by pipetting 1 μL onto a 6-mm carbon disk or by direct injection with a micro-dispenser. Hair was analyzed by dropping 1-cm segments onto the carbon bed. Breath was analyzed by trapping constituents on a bed of activated carbon.

Data were collected on the average concentration, the range and distribution of mercury in the samples. Data were also collected illustrating individual variations in mercury concentrations with time.

Concentrations of mercury found were significantly higher than values reported in the literature for a "normal" population. This is attributed to the increased accuracy gained by eliminating pretreatment steps and increasing atomization efficiency.

Determination of the exact chemical form of mercury in these samples was attempted. A dual-stage atomizer was employed. The first stage, a platinum wire loop, was gradually heated to vaporize different compounds of mercury at their characteristic temperatures. The second stage, the carbon bed atomizer, was maintained at 1450°C to atomize the vaporous metallic species. Absorption traces were obtained for various solutions of pure and complexed mercury compounds. Absorption traces of biological fluids were also obtained. Differences were observed in the absorption-temperature traces of various compounds. The utility of this technique for studying complexation was demonstrated.

GENERAL INTRODUCTION

1. Mercury in the Environment

a. History and Chemical Properties of Mercury

Mercury and its compounds have fascinated, aided and threatened mankind for centuries. Elemental mercury was found in an Egyptian tomb of the fifteenth or sixteenth century B.C.[1] The ancient Greeks described the process of heating cinnabar (mercuric sulfide) and cooling the vapor to recover "hydrargyrum" or "liquid silver", but ancient Greek physicians also warned of the poisonous nature of mercury.[2] The cinnabar mines at Almaden, Spain, provided the Romans with thousands of pounds of the red ore per year for use as vermilion paint pigment and rouge.[3] Mercurialism was one of the first recognized industrial diseases, for the Romans sent only slaves and condemned criminals to work the Almaden mines.[4,5] Mercury was used as a medicine in ancient India and China, and an ancient Chinese map depicted the oceans and rivers as elemental mercury.[2] Medieval alchemists like Paracelsus believed that all metals began as liquid mercury and that mercury could be transmuted into other metals by application of fire and the magical philosopher's stone.[6] Mercury was used from the fifteenth century until the early twentieth century as a treatment for syphilis. The treatment often resulted in severe mercury poisoning of patient and physician.[4]

Mercury has unique chemical and physical properties. It is the only metal which is liquid below 0°C and is the only element, other than bromine, liquid at 25°C.[4] Mercury vapor and the noble gases are the only elements which are monatomic at 25°C.[4] Mercury has a high vapor pressure (173 mPa at 20°C), high density (13.5 g/cm at 20°C), high surface tension, high thermal conductivity and low electrical resistivity.[7] Elemental mercury is remarkably soluble, for a metal, in polar and nonpolar solvents. Its solubility in water is about 0.6 ppb[8] and in benzene, 2 ppm.[7] At ordinary temperatures, mercury does not react with air. On heating, mercury will first absorb oxygen from the air and then release it. This reaction was used by Lavoisier and Priestley in early studies of oxygen.[8] The outer electronic configuration of mercury is $5d^{10}$ $6s^2$, and mercury exists in 2+, 1+ and low partial oxidation states such as 0.33+. Its chemistry differs significantly from that of its homologs, Zn and Cd. Unlike most metals, mercury forms covalent rather than ionic bonds; for example, the halide salts are practically non-ionized and alkylmercury compounds are not affected by water, air, weak acids and bases.[7] The stability of the mercury-carbon bond is due mainly to the low affinity of mercury for oxygen.[7]

b. The Biogeochemical Cycle of Mercury

i. Sources

Mercury is found principally in the form of cinnabar, mercuric sulfide, in Spain, Yugoslavia, Italy, California, Mexico

and Peru.[2,4] Mercury is present in fossil fuels and burning of coal and oil is an important source of mercury in the environment.

The total release of mercury into the environment from human sources has been estimated at 20×10^3 metric tons/yr.[4] For the year 1973, total mercury losses to the environment in the coterminous USA have been estimated to be 1525×10^6 g from industrial sources, 46×10^6 g from sewage and 1019×10^6 g from natural sources.[9]

Mercury and its compounds are used as catalysts, in electrical apparatus, laboratories, industrial instruments and pharmaceuticals.[2,9,10] Mercury compounds, especially organo-mercury compounds, are used as fungicides and bactericides in paint, cosmetics, household products and grain and seed dressings.[2,10] Over one-half of the total world consumption of mercury is used in the production of chlorine and caustic soda by the mercury cell electrolysis of brine.[2,10] Consequently, many of the products and chemicals prepared from chlorine and caustic soda can be contaminated with mercury. Chlorinated drinking water is just one example.

Mercury can enter the environment from all these human sources as well as from natural emissions. Raised atmospheric mercury levels have been found in industrial areas from fossil fuel burning and in rural areas from use of mercurial fungicides.[4] Mercury pollution of water has occurred extensively from industrial discharges (in Japan, Canada, and the U.S.), from

agricultural runoff (in Scandanavia) and from mercury slimicides used in wood pulp and papermaking (in the U.S. and Scandanavia). Louisiana is considered to be one of the "hot spots" of mercury emission in the U.S., with 84% of the mercury in water and 50% of the mercury in air due to man-made discharges.[9]

ii. Chemical Forms and Transformation

Metallic mercury, inorganic and organic mercury compounds are released into the environment from natural and man-made sources. These compounds can be vaporized from the earth's surface and redeposited by rain. All forms of mercury can be transformed chemically and biologically in the environment, giving rise to a "biogeochemical" mercury cycle.[7]

Metallic mercury is oxidized in water to mercuric ion. Mercuric ion can undergo a variety of reactions. It can be precipitated as mercuric sulfide, especially in anaerobic environments.[4] It can be reduced to mercurous ion or metallic mercury, a process which can be aided by bacteria.[11] Mercurous ion can disproportionate to give mercuric ion and metallic mercury. Aryl and alkoxyalkyl mercury compounds decompose to release inorganic mercuric ion.[4]

Mercuric ion can be methylated biologically to form monomethyl or dimethylmercury. This reaction is especially important because alkylmercury compounds are much more toxic than inorganic mercurials.[12] Methylation of mercury by bacteria may occur in sediments of fresh or coastal waters under both aerobic and

anaerobic conditions.[12-15] Methylation is believed to involve reaction of the mercury with Vitamin B_{12}, a methyl donor, and the pathways responsible for methionine biosynthesis.[12] Monomethyl mercury is formed first and may be converted to dimethylmercury, which is volatile and diffuses readily into the atmosphere.[4] Monomethyl mercury in water is most likely to be absorbed and concentrated by fish, since it is extremely liposoluble and not easily excreted.[10] Levels of more than 1 mg methylmercury/kg fish have been found.[4,10] Biological accumulation of methylmercury in fish may lead to the accumulation of methylmercury in animals higher up in the food chain.[10]

iii. Normal Levels of Mercury in the Environment

"Normal" levels of mercury in the environment are difficult to determine because of the ubiquitous nature of mercury and its many sources.[2] In many areas, man-made discharges have raised mercury levels far above natural levels.[2] Sample collection and analytical methods can also drastically affect the measured mercury content of environmental samples (vide infra). The following data[2] give some idea of mercury levels found throughout the world: 20-150 ppb Hg in normal soil (mean = 70 ppb); 2-10 ng Hg/m^3 in air; 0.05-0.48 ppb Hg in rainwater (mean = 0.2 ppb); 0.01-0.1 ppb Hg in normal fresh surface and ground water (mean = 0.04 ppb); and 0.005-5.0 ppb in ocean water (mean = 0.1 ppb).

The average daily intake of mercury for a non-occupationally-exposed population depends on the extent of mercury contamination in the local area. Estimates range from about 2 μg Hg/day in unpolluted areas to about 50 μg Hg/day in industrial areas, with 1-30 μg Hg from air exposure, 0.1-1.4 μg Hg from water and 1-20 g Hg from food.[4,10]

c. Toxicity of Mercury

The toxic effects of mercury depend upon the chemical form of the mercury and the route of exposure.[10,16] The most common routes of exposure are inhalation, ingestion and absorption through the skin. Mercury vapor is extremely volatile and lipid soluble. It is readily absorbed across the alveolar membrane when inhaled.[10] Elemental mercury is not absorbed from the gastro-intestinal tract; in contrast, about 10% of ingested mercuric compounds and 95% of ingested methylmercuric compounds are absorbed.[10]

In the body, mercury accumulates in the brain, kidney and hair. The biological half-life varies with the organ considered and the chemical form of mercury in question. Estimates of the whole-body half-life of mercury range from 58 to 190 days.[17-20] Mercury acts as a potent enzyme inhibitor, protein precipitant and corrosive agent. It has great affinity for sulfhydryl, phosphoryl, carboxyl, amide and amine groups.[5]

The characteristic features of mercury vapor poisoning are gingivitis, salivation, tremor and erethism.[10] Erethism is a

mental disturbance peculiar to mercury poisoning and is characterized by abnormal shyness, blushing, indecision, over-reaction to criticism and weeping.[4] Acute poisoning by inorganic mercury salts can result in severe corrosion of the gastrointestinal tract, acute renal failure, and sudden death.[10] Methylmercury poisoning has been the subject of considerable research work following the massive poisonings in Minamata, Japan (1950's), Niigata, Japan (1960's), Iraq (1970's) and around the Great Lakes, U.S. and Canada (1960's).[1] In Japan and the Great Lakes area, methylmercury poisoning occurred among a fishing populace who consumed contaminated fish. Local fish had accumulated methylmercury from biomethylated inorganic mercury discharged in industrial waste. In Iraq, poisoning occurred when grain treated with alkylmercury fungicide was made into bread and eaten instead of being planted. Symptoms of alkylmercury poisoning include central nervous system disorders, impairment of motor functions, visual problems, loss of hearing, excessive sweating and salivation.[1,2,4,10]

d. Biological Indicators of Mercury Exposure

To facilitate a reliable interpretation of the effects of mercury on the human body, a biological index of exposure to mercury is needed. Mercury concentrations in biological tissues have provided one basis for clinical diagnosis of exposure to mercury. Mercury levels in blood, urine, feces, hair, nail and internal organ tissue have been shown to reflect both endogeneous

and exogeneous exposure to mercury in many chemical forms.[10]

Several factors must be considered in choosing a tissue to serve as a biological index of exposure for routine analysis. Successful biological monitoring requires establishment of normal levels of mercury in the indicator tissue. The tissue should reflect accurately both acute and chronic exposure to mercury; that is, there must be a direct relationship between exposure and mercury concentration in the tissue. The tissue should be readily available, easily collected and easily stored. The tissue should be as homogeneous as possible. The analyte should be as concentrated as possible in the tissue, so that only a few milliliters or milligrams of sample are required for analysis. Based on these criteria, it can be seen that internal organ tissue is impractical for use in routine biological monitoring.

The composition of feces is affected by many variables and varies significantly even under similar conditions of exposure. Fecal matter does not meet the requirements stated above for a good biological index of exposure.

The specimens most commonly used for routine clinical analysis of mercury concentrations in the body are blood, urine, and, to a much lesser extent, hair. The estimation of "normal" mercury levels in these tissues from published data is not an easy task. "Normal" is taken to mean not occupationally exposed to mercury. Considerable variation in normal levels of mercury in tissue has been reported and frequently the studies do not contain

enough information to allow comparison of results.[10] Difficulties arise from many sources. Mercury levels in tissues are very low (<1 ppm Hg), often near the detection limit of the analytical method employed. Mercury concentrations in a given tissue vary with the chemical form of exposure; for example, urine mercury levels reflect inorganic mercury exposure, not alkylmercury exposure.[10] Wide diurnal and day-to-day fluctuations in urine and blood mercury concentrations have been reported which were independent of exposure.[22] Unsuspected exposure to mercury may occur through cigarette smoking, dental work or medical treatment.

An international study conducted by the World Health Organization (WHO) measured mercury concentrations in whole blood and urine from non-occupationally exposed subjects in fifteen countries.[23,24] Seventy-seven percent of the whole blood samples contained less than 5 ppb Hg and 95% had less than 30 ppb. Differences were found in blood mercury levels between countries, but no differences due to age, sex, or residence (urban or rural). WHO proposed that 30 ppb be regarded as the upper "normal" limit for mercury in whole blood. Seventy-nine percent of the urine samples had less than 0.5 ppb Hg and 95% had less than 20 ppb. No difference was found with respect to age, sex or residence (urban or rural). WHO proposed that 20 ppb be regarded as the upper "normal" limit for mercury in urine. Considerable variation has been reported on mercury levels in hair of non-occupationally exposed subjects. Reported concentrations range from 0.1 to 34

ppm Hg, with mean values of 2-8 ppm Hg.[10]

2. Analytical Problems in the Determination of Mercury

Analysis of biological and environmental samples for mercury presents several problems, regardless of the analytical technique employed.[25]

The concentration of mercury in these samples is usually very low (<ppm levels). Detection of this very small amount is the end-product of a series of steps: collection, storage, sub-sampling, and chemical treatment for separation or concentration of the mercury.[25] At each step, errors can be introduced.

Reagents used in chemical treatment, even ACS-grade reagents, often contain mercury concentrations in excess of those in the sample.[5,25] Berman[5] found that reagent blanks for the cold vapor atomic absorption spectroscopy (CV-AAS) determination, prepared from ACS-grade chemicals, contained between 0.12-0.20 ppm Hg. Neutron activation analysis (NAA) of high-purity HNO_3 showed a mercury content of about 1 ppb.[26]

Loss of mercury compounds by volatilization during sample digestion, concentration and solvent evaporation is a severe problem. Samples for mercury determination should not be ashed, even with a low-temperature plasma,[27] and should not be heated without a condenser on the vessel. Incomplete solvent extraction or incomplete oxidation of the sample matrix can cause loss of mercury.[25]

Collection and storage of samples can introduce large scale contamination or loss of mercury.[28] Mercury is rapidly lost from dilute aqueous solutions. Rosain and Wai[29] observed severe losses of mercury from aqueous solutions at pH 2 and 7 in polyethylene, polyvinyl chloride and glass containers. The half-life of 26 ppb Hg stored under those conditions was 2-4 days. Acidification to pH 0.5 prevented the loss. Feldman[30] found losses of mercury (ppb level) from glass and polyethylene containers despite acidification and addition of permanganate. He obtained reliable storage of mercury solutions in polyethylene by addition of 0.05% $K_2Cr_2O_7$ and 5% HNO_3. Leaching of bottles with concentrated nitric acid for 16 hours was reported to stabilize stored mercury solutions: 85.9% of added mercury was recovered after 14 days in treated bottles versus 33.3% recovered after 14 days in untreated bottles.[26] A wide variety of preservative systems and sample containers has been studied; results are very contradictory.[28] In general, mercury can be leached into solution from the laboratory air or container materials.[26,31] Mercury can be lost to container walls by adsorption or reduced by traces of reducing agents in solution, with diffusion of mercury vapor out of the container.[26,31]

Blood collection tubes can be contaminated with mercury in the added anticoagulant or in the rubber stopper.[32] Blood collection can result in contamination from the skin or syringe. Mercury loss from blood can occur by amalgamation with the metal

syringe needle.[32]

Weiss and Chew[33] reported a 30% loss of induced activity (12% to the walls of the polyethylene vial) during irradiation for NAA of unacidified aqueous mercury solutions. Loss occurred by both adsorption and volatilization. This could cause serious errors in mercury determinations by NAA.

Biological samples are complex matrices. Matrix effects are very pronounced in some analytical techniques. The efficiency of atomic emission and fluorescence is heavily matrix-dependent. The matrix may cause direct analytical interferences. Molecular absorption of the atomic absorption light source is one example. Natural chelating agents[25] in the sample can prevent liquid-liquid extraction of mercury or reaction with a colorimetric reagent. Bacterial action and tissue decomposition may cause volatilization or a change in the chemical form of mercury during storage.[25]

Mercury can exist in several chemical forms in biological samples; in some samples, methylmercury is the predominant form.[10] The analytical method must take all the forms of mercury into account. This is a difficult task because the exact chemical form of mercury in these samples is not known.

Finally, the method employed must be sensitive enough to detect the trace mercury levels commonly found in biological samples. In principal, one just has to take a large enough sample in order to detect mercury with any analytical method. In practice, biological samples are available in limited quantities,

usually a few milliliters or milligrams, so a sensitive method is required.

3. Common Analytical Methods for the Determination of Mercury

a. General Considerations

All analytical methods in current use for the determination of mercury in biological samples require the separation of mercury from the matrix. Some of the separation methods which have been employed for mercury are solvent extraction, amalgamation, carbon adsorption, liquid absorption and ion-exchange.[25,27]

Solvent extraction is used for separating and concentrating organic mercury compounds from aqueous solution. Benzene or toluene are used to extract alkyl and aryl mercury compounds from water prior to gas chromatographic analysis.[34] Colorimetric analysis by the dithizone method requires extraction of the mercury-dithizonate in $CHCl_3$ or CCl_4.[21] Extraction efficiency is an important consideration, since mercury recovered by repeated extractions may be lost if solvent evaporation of the combined extracts is required.[25]

Amalgamation of mercury vapor with silver or gold has been used widely to trap and concentrate mercury released by combustion and reduction-aeration of biological samples. Gold or silver-coated grids, frits, beads and pure metal wools have been used.[25] Mercury has been collected from solution by amalgamation onto a silver wire or by electrodeposition onto a copper cathode.[35,36] Amalgamated mercury is released (usually into an atomic absorption

cell) by heating.

Carbon adsorption has been used to trap and concentrate mercury released by the reduction-aeration procedure.[37] Volatilized mercury has also been collected in acidic permanganate solution.[38]

Ion exchange occurs as the result of the relative affinity of a given ion-exchange resin for particular ions. Anion exchange resin-loaded filter paper has been used to remove mercury from water for neutron activation analysis.[39] Mercury collected on a chelating resin was so strongly retained that it could not be eluted.[25] Extensive reviews of ion-exchange methods for inorganic and organic mercurials were published by Fishbein.[40,41]

Specific analytical techniques are covered in the following sections.

b. Gravimetric and Micrometric Methods

Numerous methods have been used for the gravimetric determination of mercury.[42] Electrodeposition of mercury as metallic mercury and precipitation of Hg(II) as the sulfide are two of the most useful techniques.[42] For electrodeposition of mercury metal a gold or gold-plated Pt cathode is used. About 18 hours of electrolysis is required. Washing and drying of the deposit must be done carefully to avoid loss of mercury by volatilization. The detection limit is about 50×10^{-6} g Hg and silver interferes. Even with careful handling, electrodeposition usually gives low results. Precipitation of mercury as mercuric

sulfide is useful because the sulfide is extremely insoluble; solubility losses are negligible. The precipitate is often contaminated with anions such as sulfide and many metals may interfere (Al, Cu, Cd, Zn, Sn, Tl). Precipitation techniques are accurate for microgram levels of mercury but are very time-consuming. The samples must be in solution and in some cases (e.g., sulfide precipitation) the mercury must be in a specific valence state. This requires digestion, chemical manipulation and concentration of biological samples with the risk of mercury loss and contamination.

Micrometric determination of mercury was introduced by Raaschou[43] and a variation was used extensively by Stock44 and coworkers to determine ppm levels of mercury in biological samples. The method consisted of electrodeposition of mercury onto a wire cathode, heating of the wire to volatilize mercury and collection of the condensed mercury in a capillary. The condensate is united into a globule and the diameter of the globule measured under a microscope. At 20°C, the weight of mercury, (w), in micrograms, is related to the diameter, (d), of the droplet in microns by the formula

$$w = 7.09 \times 10^{-6} d^3 \qquad (1)$$

The method is specific for mercury and was the recommended method for determination of microgram levels of mercury until the mid-1960's.

c. Colorimetric Methods

The reaction of mercuric ion with dithizone to form an orange-colored complex in $CHCl_3$ was the primary method for the determination of mercury in biological materials until the development of atomic absorption spectroscopy in the 1960's.[5,10] Other colorimetric reagents useful for mercury are di-β-naphthyl-thiocarbazone, diphenylcarbazide and phosphomolybdic acid.[42]

A number of metals interfere in the analysis, such as Ag, Au, Cu, Pd, Pt(II) and Bi.[10,42] Copper is especially a problem in biological samples. The interferences can be masked with EDTA or SCN^-.[42] Biological samples must be wet-digested, with the risk of mercury loss through volatilization. The mercury dithizone complex is light-sensitive, so the colorimetric measurements must be made rapidly. The detection limit is about 0.5×10^{-6} g Hg with a precision of about 5%.

Colorimetric analysis is inexpensive, simple and more rapidu than the micrometric method. The main disadvantages are the lack of specificity and sensitivity and the considerable amount of skilled manual work needed for each analysis. The chances of human error, mercury loss and contamination are great.

d. Electrochemical Methods

Polarography,[42] anodic stripping voltammetry,[45] ion-selective electrodes,[46] and amperometric titration[47] have been used to determine mercury in solution. Gas porous electrodes have been used to detect mercury in the gas phase by pneumato-

amperometry.[48,49]

Hg(I) and Hg(II) give well-defined diffusion currents at the dropping mercury electrode and at platinum microelectrodes.[42] Polarography can be carried out in complexing or non-complexing media. In the first case, silver and iron interfere; in the second case, oxygen interferes. The characteristics of the mercury wave have been studied by Kolthoff and Miller.[50]

Anodic stripping voltammetry can be performed, but not, of course, at the usual mercury drop or mercury film electrode. A gold-plated glassy carbon ring disk electrode[45] has been used to determine 0.01 ppb Hg in solution.

Ion selective electrodes (ISE) have been used to determine mercury directly and indirectly. A silver-glass ISE was used to determine mercury by titration with dithiooxamide.[51] A novel Hg(II) responsive ion-exchanger based ISE has been described.[52]

Gold gas-porous electrodes[49] and gas-permeable polymer electrodes have been used to detect mercury in the gas phase. Standard techniques, such as the method of Hatch and Ott,[53] are used to reduce mercury in solution to metallic mercury and to evolve mercury vapor. The mercury vapor is detected by oxidation at the gas-porous electrode. These electrodes can detect about 10×10^{-9} g Hg.

The main disadvantage is that biological samples must be dissolved prior to analysis.

e. Neutron Activation Analysis

Neutron activation analysis (NAA) is a very sensitive technique for the determination of mercury in biological samples. No pre-treatment of the sample is necessary, so solid samples can be analyzed easily. The sample is sealed in a quartz or polyethylene vial[10] and irradiated with neutrons. The gamma radiation emitted by the radioactive ^{197}Hg, which is formed, is measured. Instrumental NAA, the nondestructive analysis of the intact irradiated smple, has been used for mercury determination.[10,54,55] The detection limit is about 2×10^{-8} g Hg.[10] Biological samples contain many easily activated elements, such as sodium. Intense emission from these elements often overlaps the emission of mercury. Samples must either be allowed to decay for several days to remove interfering emissions or the radioactive mercury must be separated from the matrix (destructive analysis). Destructive analysis generally involves wet-digestion of the sample followed by precipitation, chelation extraction or adsorption of the mercury.[10,56] Destructive analysis gives better sensitivity than instrumental analysis, but at the risk of mercury loss or sample contamination.

The detection limit for destructive NAA is about 2×10^{-10} g Hg.[10] Neutron activation is a precise (10%), sensitive method for total mercury determination in biological samples. The main disadvantage is that a neutron source, generally a nuclear reactor, is required and may not be readily available. Reports

have also been published detailing loss of mercury by adsorption onto or diffusion through the sample vials during the irradiation and decay periods. This could result in erroneously low determinations.[33]

f. Atomic Emission

The atomic emission method is based on the excitation of elements in a sample by an electrical discharge or plasma. The excited atoms emit light of characteristic wavelengths. Mercury has been detected at the ppb level using microwave-excited plasmas.[57,58] The advantage of atomic emission is that it can be used for the simultaneous determination of numerous metals.[59] Disadvantages include severe self-reversal of the mercury resonance lines, high background radiation from the sample and matrix effects from the sample. Direct spectral interference (overlap of emission lines of two or more elements) is a definite problem in emission analysis of complex biological matrices. Some of these disadvantages have been overcome by using an emission detector after gas-chromatographic separation of mercury compounds.[57] The mercury resonance line at 253.7 nm is usually the wavelength measured.

g. Atomic Fluorescence

Atomic fluorescence is based on excitation of atoms by using radiation from an intense line source such as a laser or electrodeless discharge lamp. Excited atoms reemit characteristic wavelengths which are measured at right angles to

the light source. Atomic fluorescence has been employed for the detection of mercury, principally with microfurnace or carbon filament techniques.[27] Reduction-aeration and wire filament atomizers have been used.[60,61] The detection limit for mercury by atomic fluorescence is about 1-15 ng Hg and the precision is 5-10%.[60,61] Organic vapor interference is claimed to be lower than in atomic absorption measurements but particle scattering effects are higher.[27] Atomic fluorescence analysis compares well with atomic absorption analysis[60,61] and can be performed without a hollow cathode lamp and monochromator.

h. Atomic Absorption

Monatomic mercury vapor was the first element to be determined by atomic absorption.[62] Atomic absorption spectroscopy (AAS) is the most widely-used technique for the determination of mercury in biological materials because of its specificity, simplicity, sensitivity, ease of operation and low cost.

Flame atomizers are not often used for the atomic absorption determination of mercury. The sensitivity is about 10 ppm, which is not sufficient for the analysis of biological and environmental samples.[63] Also, traces of reducing agents in samples can reduce Hg^{2+} and Hg^{1+} to Hg°. The atomization efficiency of Hg^{2+} (in calibration standards) is less than that of Hg° and an error would result.[63]

Almost all atomic absorption techniques in current use are variations of the cold-vapor atomic absorption (CV-AAS) method.

The mercury in the sample is converted to mercury vapor which is pulled into a quartz gas cell placed in the light path of an atomic absorption spectrometer.[10,25,27] The essential difference among the methods is the way in which the mercury is converted into the elemental vapor phase. Two major conversion methods are in common use. They are chemical reduction in solution followed by aeration to release the mercury vapor and volatilization of mercury by heat.[25]

Reduction-aeration methods are based on the work of Hatch and Ott[53] and literally hundreds of variations have been reported. Samples are usually treated with a mixture of sulfuric acid, potassium permanganate and potassium persulfate to oxidize all mercury to mercuric ion. Mercuric ion is then reduced to mercury metal by addition of $SnCl_2$. Air is bubbled through the solution and passed through the atomic absorption cell. Reduction-aeration methods can estimate the amount of organic mercury in a sample by determining separately total mercury and inorganic mercury. Inorganic mercury only can be determined by not oxidizing the sample, because Sn(II) will not reduce organically-bound mercury. The accuracy of the reduction-aeration technique for total mercury determination depends on the complete destruction of organic material by the wet digestion-oxidation step. Many acid and oxidant combinations have been used.[27] Digestion procedures differ in the time and temperature used as well as what kind of condenser (if any) was used.[27] Interferences in the reduction-

aeration procedure have been reported. Depressive interferences are caused by nitrate, perchlorate, sulfate, phosphate, sulfide, thiosulfate, bromide, iodide, Pd, Pt, Au, Ag, Cu, and Zn.[27] These and other matrix interference effects can be reduced by the use of standards of similar composition to that of the samples. Molecular absorption by volatile organic vapors can be accommodated by background correction. Drying tubes are usually placed between the sample vessel and the absorption cell: these can cause contamination or loss of mercury.[27] Foaming of aerated solutions can cause low mercury values.[27]

Combustion and pyrolysis techniques eliminate many of the problems associated with the reduction-aeration method and provide a one-stage process for the determination of mercury. Oxidizing furnaces, nebulizer burners, carbon and graphite rod or tube atomizers and wire loop or metallic ribbon atomizers have been used to release mercury vapor from samples.[27] The chief difficulty with these techniques is molecular absorption by volatile species and light scattering losses produced by smoke and particulates. This vapor-phase interference can be accommodated by measurement of the molecular absorption[27] with a hydrogen lamp, Zeeman-split mercury electrodeless discharge lamp, or the wings of a pressure-broadened mercury vapor lamp. Another method of correction is to collect the mercury from the vapor phase, by amalgamation, for example, before the determination.

The spin-forbidden resonance line at 253.7 nm is used most often for the determination of mercury. The spin-allowed resonance line at 184.9 nm has a much greater oscillator strength, but is not often used because of the difficulty in working in the vacuum-ultraviolet region.[63,64] Direct spectral interference is not as great a problem in AAS as in atomic emission spectroscopy, but cobalt weakly absorbs at the 253.7 nm line.[63] A 1000 ppm Co solution results in approximately 10% absorption. This normally would not be a problem because Co levels in most biological samples are less than 1 ppm.

Another problem with the determination of mercury by AAS arises in the light source. Atomic absorption line widths are very narrow (on the order of 0.002 nm), especially at short wavelengths like the mercury resonance lines, and a continuous light source cannot be used to measure them.[63] This problem was overcome by Walsh,[65] who introduced the hollow cathode lamp (HCL). A hollow cathode lamp emits very narrow spectral lines which are completely available for absorption, in most cases. But for mercury, and several other volatile metals, free atoms sputtered from the cathode form an atom cloud inside the lamp.[63] This atom cloud is able to reabsorb some of the radiation emitted by the cathode. This absorption, or self-reversal, occurs at the very center of the emission line and results in decreased sensitivity and distorted calibration curves.[63] Self-reversal can be alleviated by using a demountable HCL, in which the atom cloud is

removed by continuous pumping of filler gas, in place of a commercial sealed HCL.

In this research, a method was developed for the direct determination of mercury in biological samples using a unique carbon bed atomizer for atomic absorption spectroscopy which reduced the interferences while retaining the advantages of a one-step process. A specially-designed demountable HCL was used as the light source.

The historical development of AAS is briefly discussed in the next section.

4. Historical Development of Atomic Absorption Spectroscopy

The first experimental investigation of the phenomenon of atomic absorption by mercury vapor was made about 1910 by Wood.[66] The first practical use of atomic absorption for the determination of mercury in air was made by Muller,[67] Pringsheim[68] and Woodson[62] in the 1930's. Atomic absorption spectroscopy for metals other than mercury was introduced by Walsh[65] in 1955. The technique is based on the absorption of a characteristic frequency of light (the resonance frequency ν) by free atoms of the element of interest. The factors affecting the total amount of absorption are expressed in the fundamental equation of atomic absorption (Equation 2).[63]

$$\int_0^\infty K_\nu \, d\nu = \frac{\pi e^2}{mc} Nf \qquad (2)$$

where:

$\int K\nu d\nu$ = total absorption over the absorption band

e = charge of an electron

m = mass of an electron

c = speed of light

N = total number of atoms that can absorb the frequency of light

f = oscillator strength at frequency ν

It can be seen from the above equation that, for a given frequency ν, the amount of absorption depends only on N, the number of free atoms available which absorb frequency ν. The oscillator strength, f, is the absolute limiting factor for the sensitivity of atomic absorption, but the size of the absorption signal is determined by N, the number of free atoms produced in the atomizer. The efficiency of production of free atoms determines the practical sensitivity and usefulness of the technique.

Until the late 1960's, flames were used almost exclusively to atomize samples. The sensitivity of flame atomizers[69] was approximately 10^{-7} g. This was not sensitive enough for analysis of most environmental and biological samples. Theoretical calculations[70] showed that approximately 10^{-16} g of an element should give a 1% absorption signal. Obviously, greater atomization efficiency was needed to improve the sensitivity of the method. A significant breakthrough in atomic absorption occurred in 1961 when L'vov[71] developed the carbon atomizer. Carbon

atomizers demonstrated much greater sensitivity than flames, on the order of 10^{-11} g for 1% absorption, but suffered from a lack of precision and accuracy. The situation changed in 1969, when two new designs in carbon atomizers were presented at the International Atomic Absorption Conference in Sheffield, England, one by West[72] and the other by Robinson.[73] Another modification was proposed by Massman.[74] The West and Massman atomizers were subsequently adapted for use in commercial atomic absorption spectrophotometers.

Commercial carbon atomizers (also called graphite furnaces) operate by the passage of a high current through the carbon piece, causing resistance heating which decomposes and atomizes the sample. Incomplete degradation of the sample matrix causes considerable interference due to molecular absorption. Commercial graphite furnaces especially suffer from this problem since decomposition and atomization take place within the light path. Therefore, a three-step atomization program is used in most commercial spectrophotometers. The first step is evaporation of the solvent at a low temperature (approximately 100°C). This is followed by an ashing step at a medium temperature (300°-500°C) to decompose the sample matrix. Finally, atomization occurs as the atomizer is rapidly heated to about 2400°C. Absorption is recorded during this final step, always with simultaneous background correction. Although this method requires precise programming of the time and temperature cycles, the technique has

been developed to a high degree of reproducibility.[69]

The major drawback to this atomization process is that significant losses of volatile metals such as mercury occur during the drying and ashing steps, causing low results.[63]

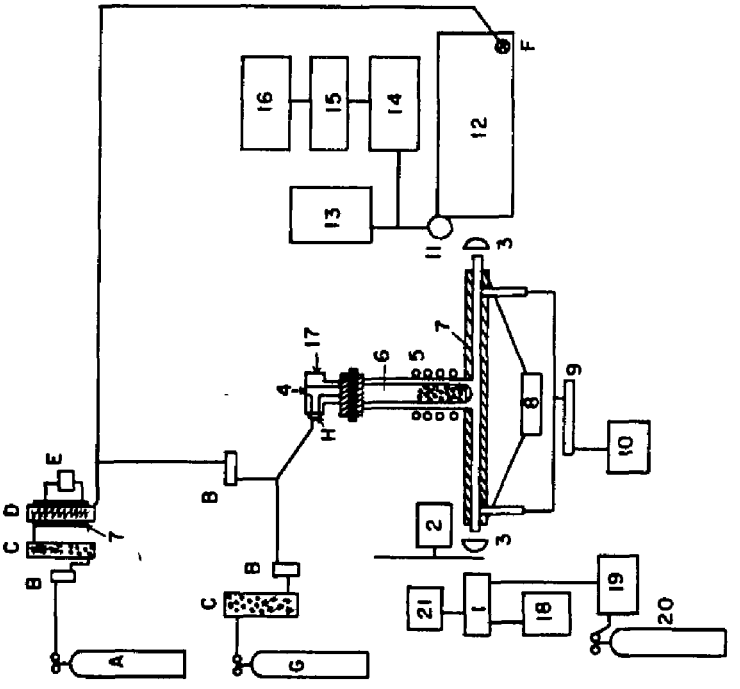
5. Advantages of the Quartz "T" Atomizer and Demountable Hollow Cathode Lamp

The problem of vaporization losses before atomization is avoided and molecular background absorption is greatly reduced in the Robinson carbon bed atomizer (the quartz "T"), illustrated in Figure 1. This is the atomizer design used for the research reported in this dissertation. The atomizer consisted of a quartz "T"-shaped absorption cell. The vertical stem of the "T" was fitted with a quartz inner sleeve packed with carbon pieces. The carbon bed was heated to about 1450°C by coupling to a radio frequency (rf) generator: this was the site of sample degradation and atomization. The light path was the cross-piece of the "T". It was separated from the atomization site and was heated to about 1000°C by resistance heating. A vacuum pump connected to exit ports from the light path maintained a constant air or nitrogen flow through the bed. A sample introduced onto the carbon bed was thus drawn through the bed at a slow rate compared to commercial graphite furnaces. Complete decomposition and atomization occurred before the sample elements were drawn into the light path. All of the sample elements had to exit through the light path and absorption was monitored continually; thus, volatiliza-

FIGURE I

BLOCK DIAGRAM OF ATOMIC ABSORPTION SYSTEM

FOR WORK IN VACUUM ULTRAVIOLET REGION



1. HOLLOW CATHODE LAMP
2. CHOPPER
3. PLANO-CONVEX LENS
4. SAMPLE INLET
5. RF COILS
6. ATOMIZER CELL WITH CARBON BED
7. ASBESTOS-WRAPPED NICHROME WIRE
8. VARIAC
9. FLOW METER
10. AIR PUMP
11. PHOTOMULTIPLIER
12. MONOCHROMATOR
13. POWER SUPPLY
14. AMPLIFIER
15. POTENTIOMETER
16. RECORDER
17. BRASS INLET CAP
18. VACUUM PUMP
19. FLOW CONTROL
20. ARGON TANK
21. POWER SUPPLY

A. NITROGEN TANK
B. FLOW METER
C. ACTIVATED CHARCOAL/ SILICA GEL SCRUBBER
D. COPPER TURNINGS
E. VARIAC
F. INLET VALVE FOR N_2
G. OXYGEN TANK
H. PURGE GAS INLET

tion losses were avoided.

Oxygen reacts with the carbon bed according to the following equations.[75]

$$O_2 + C \rightarrow CO + CO \quad (3)$$

$$CO_2 \xrightarrow{900°C} CO + O \quad (4)$$

At temperatures greater than 900°C, Equation 4 was favored, producing an abundance of CO in the carbon bed. This provided a reducing atmosphere in the atomizer in which organic compounds were reduced to CO and H_2. These compounds do not absorb excessively in the ultraviolet (UV) region. Molecular background absorption was minimized so effectively that analyses could be carried out at the 184.9 nm Hg resonance line in the vacuum-UV region, as well as at the 253.7 nm Hg resonance line. Metal compounds were reduced to their atomic states very efficiently, as was demonstrated by the fact that sensitivity for most elements was on the order of 10^{-12} g.[63]

Another improvement in sensitivity was due to use of a unique demountable hollow cathode lamp. Self-reversal within commercial sealed lamps is a severe problem with volatile elements like mercury. A demountable HCL reduced the amount of self-reversal because the sputtered atom cloud is pumped out of the lamp. A further refinement to the demountable HCL was made by Binder,[76] a

former member of this research group. In his design, the filler gas is forced to exit at the back of the cathode, so the sputtered atom cloud is removed from the light path very efficiently. The flow-through demountable HCL is illustrated in Figure 2.

It can be seen that the Robinson carbon bed atomizer fulfilled several requirements for the direct determination of mercury in biological samples. The long contact time of the sample with the carbon bed under a reducing atmosphere significantly reduced the molecular absorption caused by the complex organic matrix. Volatilization losses were eliminated by the flow-through atomizer design. The sensitivity of the technique was sufficiently low to detect mercury at the ppb level.

It was the objective of this research to develop techniques which applied the advantages of the carbon bed atomizer to the direct determination of mercury in several types of biological samples. Part I of this dissertation describes the direct determination of mercury in water, urine, sweat, hair, breath, saliva, whole blood and serum. Analysis of these various specimens helped to elucidate the mercury balance in the body and the pathways for excretion of mercury from the body.

6. Metal Speciation

The toxicity of mercury depends both on its concentration and chemical form. The chemical form of mercury in the body is the key factor which governs its absorption, transport, biotransformation, retention and excretion. Alkyl mercury compounds are many

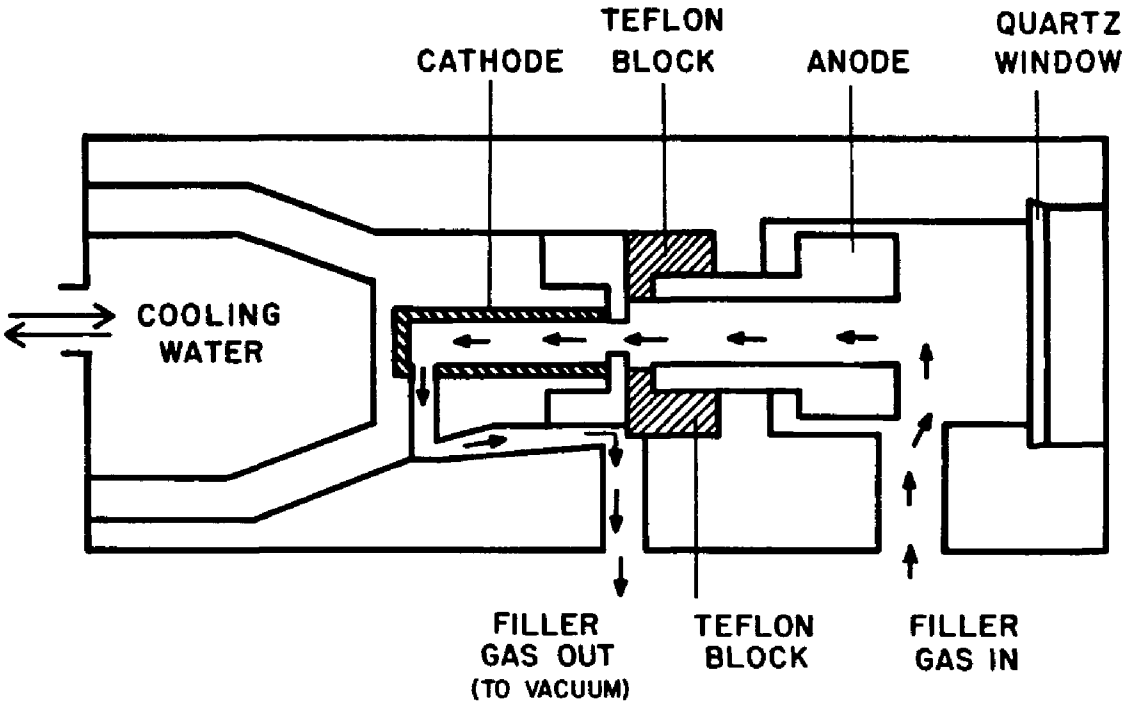


FIGURE 2: FLOW-THROUGH DEMOUNTABLE HOLLOW CATHODE LAMP

times more toxic than aryl or inorganic mercurials. For example,[77] CH_3Hg^+ can penetrate the hydrophobic core of a protein more easily than Hg^{2+}. CH_3HgCl is a better inhibitor of membrane-bound enzymes than inorganic mercury. More than 70% of ingested CH_3HgCl is absorbed by the human body while only 5% of ingested $HgCl_2$ is absorbed.[79] The very hydrophobic dimethylmercury is found concentrated almost entirely in lipids and appears to behave as a chemically and physically inert substance until ionized.[80,81] Elemental mercury vapor is a non-polar, highly diffusible gas which is soluble in lipids. This leads to rapid and complete transfer through the lungs into the bloodstream and rapid diffusion across the blood-brain barrier.[82,83]

The mechanisms by which mercury and its compounds exert toxic effects are not known, but it is most probable that the metabolism of mercury in the body depends on its chemical form. Speciation of chemical form has also become important in the study of synergistic effects of groups of elements.

It is apparent that analytical techniques are needed which provide not only total metal determination, but also differentiation between metal compounds. Analytical techniques such as infrared and ultraviolet absorption, nuclear magnetic resonance and X-ray spectrometry can differentiate between metal species but do not possess the requisite sensitivity for biological materials.

The methods which have evolved for trace mercury speciation almost all rely on a chromatographic technique for separation with a determination of the mercury content of each fraction. Gas

chromatography with an electron capture detector was introduced by Westoo[84,85] for the determination of alkyl mercury in fish. Many variations of the technique have been published,[25] but the method generally involves conversion of organic mercury compounds to organomercuric halides which are subjected to gas chromatographic analysis. The halide (not the mercury) is detected by the electron capture detector. One source of error in this method is that some mercury compounds decompose on the column to diphenyl- or dimethylmercury.[25] These compounds are not detected with an electron capture detector.

Gas chromatography has been coupled with several different metal-specific detectors for mercury speciation. Microwave emission, atomic absorption, atomic fluorescence and mass spectrometers have been used in place of conventional chromatographic detectors.[25] Although such coupled techniques for mercury speciation have been successful, the use of two techniques introduces additional sources of error. Extractions and separations are never complete and the problem is aggravated at ppb levels. Loss or contamination of the sample or changes in chemical form of the sample can occur during separation stages. The development of a spectroscopic method which could accomplish both separation and detection of metal compounds would offer a distinct advantage in speciation studies.

Part II of this dissertation describes the development of an experimental technique which used AAS to distinguish between

different chemical forms of mercury. The technique made use of a two-stage atomizer. The first stage was gradually increased in temperature to volatilize selectively various chemical forms of mercury. The second stage was maintained at 1450°C to atomize the vaporized mercury compounds. The volatilization of pure mercury compounds and mercury in biological samples was studied.

7. Summary

The following topics will be covered in this dissertation:

Part I: The Direct Determination of Mercury in Environmental and Biological Materials

Chapter 1: The Direct Determination of Mercury in Water

Chapter 2: The Direct Determination of Mercury in Urine

Chapter 3: The Direct Determination of Mercury in Sweat

Chapter 4: The Direct Determination of Mercury in Whole Blood and Serum

Chapter 5: The Direct Determination of Mercury in Hair

Chapter 6: The Direct Determination of Mercury in Breath and Saliva

Part II: Speciation of Mercury Compounds

Chapter 7: Speciation of Mercury Compounds by Differential Volatilization-Atomic Absorption Spectroscopy

PART I

THE DIRECT DETERMINATION OF MERCURY IN ENVIRONMENTAL AND BIOLOGICAL MATERIALS

CHAPTER 1

THE DIRECT DETERMINATION OF MERCURY IN WATER

A. INTRODUCTION

Ingestion of mercury present in drinking water and food is the major route of exposure to this metal for a "normal" (non-occupationally exposed) population. Therefore, it is important to monitor the amount of mercury in drinking water, so that this exposure can be controlled.

1. Mercury Concentrations in Water

Numerous studies have been conducted on mercury concentrations in natural and finished (i.e., treated, ready to drink) waters around the world. Groundwater has been found to contain 0.01-0.10 ppb Hg.[2,9,85] Surface waters such as lakes, rivers, and reservoirs have been found to contain 0.01-17 ppb Hg.[2,9,85] Concentrations of mercury greater than 1 ppb in surface water have occurred mainly in small streams or in industrially-contaminated areas. A nationwide survey by the U.S. Department of the Interior in 1970 showed that only 4% of surface water sources had mercury concentrations in excess of 1 ppm; most contained between 0.1 and 1.8 ppb.[85] Industrial contamination has been shown to raise mercury levels in water dramatically; for example, water near a battery plant in Michigan was found to contain 1000 ppm Hg.[86] Mercury levels of 0.03-2 ppb have been measured in seawater,[2,9,85] while concentrations of 0.05-0.5 ppb have been found in rainwater.[2,16]

Limits have been set on the amount of mercury allowed in drinking water, which, in the U.S., usually means in finished tap water. The World Health Organization limit was set at 1 ppb,[87] while a U.S. Environmental Protection Agency (EPA) limit of 2 ppb was established.[85] An EPA survey of 273 water supplies across the country found that 261 contained $\leq$1 ppb Hg, 11 contained between 1 and 4.8 ppb Hg and one contained >5 ppb Hg.

While mercury levels in "normal" water supplies generally have been found to be below 1 ppb, they can vary a great deal due to factors such as pH, temperature, the redox potential of the system and the presence of natural chelating or complexing agents. These factors may increase greatly the amount of mercury in water. Mercury concentrations in water may be increased further by the activities of man. Anthropogenic input has been estimated to have increased mercury levels in rivers fourfold.[9] Chlorine, used for purification of tap water, can be a source of mercury. Most chlorine is produced by mercury cell electrolysis of brine and so can be contaminated with mercury.

The contribution of drinking water to the body burden of mercury in an individual can be calculated. If it is assumed that drinking water contains 2 ppb Hg and that an individual consumes 2 L of water per day, 4 µg Hg/day is ingested in this way.[85] This represents about 10% of the estimated daily intake of mercury.

2. The Chemical Form of Mercury in Water

Mercury is present in water as a result of anthropogenic

input and cyclic natural processes in which mercury from the earth's crust is washed into bodies of water. Mercury can be discharged into the environment as metallic mercury, inorganic mercury compounds, alkyl, aryl, alkoxy and other organic compounds.[16,85] Once in an aqueous environment, mercury compounds can undergo a variety of transformations.

Inorganic mercury can be converted to methylmercury cation and dimethylmercury by microorganisms[16,88] or by suitable chemical donors, such as tin and lead alkyl compounds.[89] Biological methylation of Hg^{2+} is believed to occur via methyl corrinoid derivatives, such as Vitamin B_{12}, since they are the only known methylating agents capable of transferring methyl groups as carbanions, CH_3^-. The product of the methylation reaction is mainly methylmercury cation, CH_3Hg^+, under neutral or acidic conditions and dimethylmercury under basic conditions.[89] The efficiency of the reaction is dependent upon pH, temperature, the presence of sulfides and complexing agents, microbial activity and other factors. Methylation by chemical donors can also occur; trimethyl lead cation has been shown to be as effective as Vitamin B_{12} in the methylation of mercury. Once formed, dimethylmercury is volatile enough to escape into the atmosphere, where it is photolyzed to give elemental mercury, methane and ethane.[88] Methylmercury cation is an unusually strong acid, with a pK_a of about 5 (equivalent to acetic acid).[90] It will react with water to give methylmercury

hydroxide, as the following reaction shows.

$$CH_3Hg^+_{(aq)} + H_2O_{(\ell)} \rightleftharpoons CH_3HgOH_{(aq)} + H_3O^+_{(aq)} \qquad (5)$$

At pH 7, the hydroxide form is very much favored and the cation is a relatively unimportant species.[99] However, CH_3Hg^+ is a very soft acid and binds strongly to soft bases. The chloride, bromide and iodide compounds of CH_3Hg^+ exhibit high stability and low aqueous solubility. CH_3HgX (X = halide ion) compounds do not interact strongly with water because the principal coordination number of mercury is two.[90] Methylmercury halides do exhibit high lipid solubility and may play an important role in the transport of CH_3Hg^+ into biological systems. Methylmercury compounds can undergo biological degradation to methane and elemental mercury, but, in general, CH_3Hg^+ is not readily decomposed except by specific biochemical processes.[89]

Interconversion of the three inorganic forms of mercury in water, Hg°, Hg_2^{2+} and Hg^{2+}, can occur as a result of chemical or microbial action. Mercurous ion disproportionates to give elemental mercury and mercuric ion, as is shown.[9]

$$Hg_2^{2+} \rightleftharpoons Hg^{2+} + Hg^\circ \qquad (6)$$

Hg° has sufficient vapor pressure to escape from the aqueous environment into the vapor phase.[88] Under the reducing con-

ditions often found in sediments, H_2S is present. This can react with Hg^{2+} to form HgS (pK_s = 53). Aerobes can re-solubilize Hg^{2+} from HgS by oxidizing the sulfide to sulfate. Hg^{2+} can be reduced to Hg° by enzymes present in bacteria.[88] The Hg^{2+} ion has a strong tendency to complex formation, especially with compounds containing C, N, P and S ligand atoms.[8] Mercuric ion also forms compounds and complexes with halide ions. Species such as HgX^+, HgX_2, HgX_3^- and HgX_4^{2-} are formed (X = halide ion).[8]

Evidence indicates that the predominant form of mercury in fresh water is Hg^{2+}, present as chelates and complexes with a variety of organic and inorganic ligands. Oceanic mercury is generally present as an anionic chloride complex which does not have as pronounced a tendency to bind to particulate substances and settle out as do mercury compounds in fresh water.[85] Methylmercury can be bioconcentrated many thousandfold in fish and other aquatic organisms due to its lipid solubility and affinity for sulfur groups in protein. A simplified diagram of the major forms of mercury in water and their interconversions is presented in Figure 3.

3. Analytical Considerations in the Determination of Mercury in Water

Water is one of the most common materials analyzed in any laboratory. It is analyzed to monitor concentrations of substances in the environment. Analytical standard solutions are

TRANSFORMATION OF MERCURY IN THE ENVIRONMENT

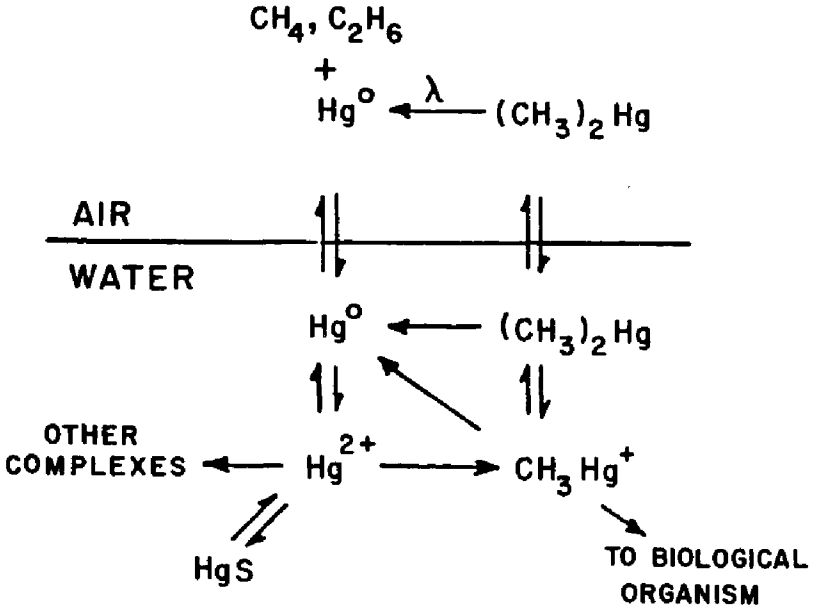


FIGURE 3: THE MAJOR CHEMICAL FORMS OF MERCURY IN WATER AND THEIR ROUTES OF TRANSFORMATION ARE SHOWN.

almost invariably aqueous. However, water is not a simple matrix and it can pose problems for even experienced analysts.[27,91] Fresh water contains numerous solid and dissolved species. A typical composition is given[92] in Table 1. Trace elements may disappear from aqueous solutions by volatilization,[93,94] absorption onto container walls,[93,95] and bacterial action.[27,96] Acidification of natural waters with nitric acid is a recommended procedure to inhibit adsorption of trace metals onto container walls.[97] Such acidification may actually increase the possibility of loss, since mercury dissolved from particulate matter may then volatilize.[98]

Mercury is present in water at trace (< ppm) levels and preconcentration of the metal is often necessary prior to analysis. Techniques such as evaporation and freeze-drying can lead to considerable losses of the element of interest.[91] Losses of mercury can occur on transfer of solutions from vessel to vessel and on filtration.[27]

Samples can be contaminated by reagents used in the chelation and extraction or reduction-aeration procedures used to concentrate mercury from water. The contamination is often insidious; for example, unstoppered bottles of acidic potassium permanganate used for many digestion procedures readily collect mercury from contaminated laboratory air.[27]

Incomplete destruction or removal of organic compounds can cause errors in analysis.[27] Failure to completely destroy the

Table 1

Composition of Fresh Water[92]

Dissolved Species	
Principal Cations:	Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}
Principal Anions:	HCO_3^{-}, PO_4^{3-}, SO_4^{2-}, Cl^{-}, SiO_3
Trace Cations:	Al^{3+}, Fe^{3+}, NH_4
Trace Anions:	NO_3^{-}, SH^{-}
Inorganic Molecules:	H_2CO_3, H_2S
Organic Molecules:	humic acid, fulvic acid, amino-acids, proteins
Inorganic and Organic Pollutants	
Solids	
Organic:	biota, degradation products, humic and fulvic acids
Inorganic:	clay minerals, metal oxides, hydroxides, carbonates, sulfides

Table 2

Absorption by 3 mm-diameter Filter Paper Disks at 184.9 nm

Disk Treatment	Number of Disks Analyzed	RESONANCE ABSORBANCE (mean + σ)	BACKGROUND ABSORBANCE (mean + σ)
Untreated	15	0.2041 ± 0.0701	0.0883 ± 0.0339
Acid washed	14	0.1965 ± 0.0560	0.0630 ± 0.0232
Acid washed and heated in atomizer	26	0.1593 ± 0.0357	0.0565 ± 0.0088

organic matrix can prevent extraction or detection of combined forms of mercury. Failure to remove organic compounds can result in molecular absorption of the resonance line during CV-AAS analysis. Mercury can easily be lost by volatilization from hot digests.

Some inorganic species can interfere with the determination of mercury in water. Iodide is known to inhibit the extraction of mercury by dithizone.[99] Depression of atomic absorption signals can be caused by nitrate, sulfate, oxalate, phosphate, sulfide, bromide, iodide, Pd, Pt, Au, Ag, Cu, Zn, Se, Te, Mo, and W, among other species.[27]

Problems with accuracy and precision plague most current methods for the determination of mercury.[91] In 1975-76, the International Atomic Energy Agency sent out simulated fresh water samples containing trace amounts of several elements to laboratories around the world for an intercomparison study.[91] The true value of the mercury level was 1 ppb; the concentrations reported by 21 laboratories ranged from 0.13-218.8 ppb. Only values between 0.13 and 3.0 ppb were accepted; the relative standard deviation of the accepted values was 39.7% (mean = 1.62 ppb). No correlation was found between the precision of results produced by a given laboratory and their accuracy. These problems were due in large part to the low levels of mercury found in water.

Speciation of mercury in water is also difficult. The concentration of each specific chemical form is very low. Speciation procedures often require adjustment of pH and selective extraction; addition of chemicals to the sample can change the nature of the mercury species present. Extraction and separation techniques are often not efficient at the ppb level.

4. Current Methods for the Determination of Mercury in Water

Methods for the determination of mercury in water can be divided into two categories, those which measure total mercury concentration and those which are capable of speciation. These two categories will be discussed separately.

a. Methods for the Determination of Total Mercury Concentration in Water

The most common methods of determining mercury in water are colorimetry with dithizone, neutron activation analysis and cold-vapor atomic absorption spectroscopy.

Colorimetric analysis with dithizone was carried out by extraction of mercury from an acidic solution with dithizone in chloroform. In an acidic medium, the only metals which interfered were copper and the noble metals.[2] Mercury dithizonate in chloroform was determined colorimetrically at 490 nm. The absorbance must be measured immediately as the mercury dithizonate complex is not stable on exposure to light. The detection limit was 0.5 μg Hg. Many variations on the procedure have been published.[2] For example, Ueno et al.[100] measured mercury

in water by mixing the water with an aqueous solution of copper dithizonate. Dual-wavelength photometry was used to measure the difference in absorbances at 507 nm and 490 nm, which was proportional to the mercury concentration. The procedure was simple and eliminated the need for a reagent blank. The disadvantages of dithizone colorimetry for the determination of mercury were numerous. Dithizone was a relatively non-specific reagent and interferences from other metals were possible.[2] Analytical conditions had to be controlled to permit the quantitative extraction of mercury. The procedure was very time consuming and required a skilled analyst.

Neutron activation analysis (NAA) has been used extensively for the determination of mercury in water.[2,101,102] Samples were sealed in quartz ampoules and irradiated in a thermal neutron flux for 2-3 days. Samples can be analyzed non-destructively by measurement of either the 77 KeV gamma radiation of ^{197}Hg (half-life = 65 hr) or the 279 KeV gamma radiation of ^{203}Hg (half-life = 47 days). A Ge (Li) detector and multichannel analyzer were used. The sensitivity of non-destructive NAA was rather poor, about 0.05 µg Hg. The sensitivity can be improved by chemically separating the mercury from the sample and interfering radioactivities. For example,[102] the contents of the irradiated ampoules can be transferred to a distillation apparatus along with concentrated nitric, sulfuric and perchloric acids. Mercury is distilled over as mercuric chloride. Mercury in the distillate

is deposited on a gold foil electrode by electrolyzing the solution for 17 hours. The gamma radiation from ^{197}Hg on the foil is measured in a NaI(Tl) well crystal with a multichannel analyzer. Neutron activation analysis with radiochemical separation was a very sensitive technique for the determination of mercury in water. The sensitivity was approximately 0.5 ppb. The disadvantages were that it was extremely time-consuming (6-30 days), expensive, and required a nuclear reactor and highly qualified personnel. Mercury can be lost from samples at normal irradiation temperatures,[2] which can result in negative errors.

Cold-vapor atomic absorption spectroscopy (CV-AAS) has been the most popular analytical method for the determination of mercury since the technique was introduced by Hatch and Ott[53] in 1968. In this reduction-aeration technique, mercury was reduced to the elemental state in solution by addition of stannous chloride. Mercury vapor was released from solution by aeration and passed through a gas cell in the light path of an atomic absorption spectrometer. Hundreds of variations on the original method have been reported and several excellent reviews are available.[2,27,103] Many combinations of acids and oxidizing agents have been used for sample digestion. Changes in the digestion procedure can be used to release selectively certain chemical forms of mercury. For example, reduction with stannous chloride after digestion with acidic permanganate has been shown

to release organic and inorganic mercury, while reduction with no prior digestion released only inorganic mercury.[27] The organic mercury content can be found by difference. Many variations have described a concentration step for trapping the released mercury vapor prior to atomic absorption measurement. The mercury vapor is amalgamated with a silver or gold trap or adsorbed onto activated charcoal and then released into the absorption cell by heating the trap. This produced a sharper, narrower signal than that produced by the conventional method.

Mercury has also been determined by vapor phase AAS following its release by heating. Electrothermal atomization in carbon or graphite microfurnaces and on wire loops and metallic ribbons has been used.[27,104,105]

Almost all atomic absorption studies of mercury have measured absorption at the spin-forbidden 253.7 nm resonance line ($6^3P_1 \leftarrow 6^1S_0$). It has been known[106] that the resonance line at 184.9 nm ($6^1P_1 \leftarrow 6^1S_0$) in the vacuum-ultraviolet region has an oscillator strength ($f = 1.18$) which is about fifty times greater than that of the 253.7 nm line ($f = 0.026$). The greater oscillator strength of the 184.9 nm should lead to increased sensitivity in AAS measurements, but the line has not been used extensively due to the difficulties encountered in working in the vacuum-ultraviolet region. Atmospheric oxygen, water vapor and products of the atomization process such as H_2 and CO have been shown to absorb in this region.[64,107,108] Robinson et al.[64] per-

formed a direct determination of mercury in air using the quartz "T" atomizer and the 184.9 nm resonance line. An increase in sensitivity of an order of magnitude over the 253.7 nm line was achieved. Tanabe et al.[107] used a long light path absorption cell similar to the crosspiece of the Robinson quartz "T" atomizer, and nitrogen purge gas to analyze aqueous solutions by CV-AAS at 184.9 nm.

The advantages of the CV-AAS technique were many. It was simple, rapid, inexpensive and more sensitive than colorimetric methods. The method also had disadvantages. Incomplete oxidation and dissolution of mercury species present in water can result in failure to detect these species. Mercury can be lost by adsorption onto the walls of the tubing and gas cell. Molecular absorption of the resonance line by volatile organic compounds can occur. The reported detection limits covered a wide range, from 0.008 to 100 ppb.[2]

Other analytical methods have been used for the determination of mercury in water, but, since these methods are not used extensively, they will be discussed only briefly.

The use of atomic fluorescence for the determination of mercury has been reviewed.[2,27,109] Mercury vapor was usually released by reduction-aeration techniques and the 253.7 nm fluorescence emission from the vapor was measured. Atomic fluorescence had several advantages over absorption methods. The equipment was simple and economical, the sensitivity was much better than that

of the absorption technique and spectral interferences were reduced in number and in severity.

Atomic emission spectroscopy with an atmospheric pressure DC argon plasma excitation source has been used for mercury determination.[110] Emission was measured at the 253.7 nm resonance line. The absolute detection limit was 60 pg Hg with a linear dynamic range of 10^3. The DC argon plasma has been used as an element specific detector for gas chromatography.[110]

Paper[111] and liquid[112] chromatography have been used to detect mercury in water. The determination of mercury in water by a variety of electrochemical methods has been reported. A commercial gold film mercury vapor detector has been coupled with reduction-aeration techniques to measure mercury in solution.[113] The evolved mercury vapor was adsorbed onto thin gold films in the detector, which caused an increase in resistance (displayed on galvanometer) proportional to the mercury concentration. A detection limit of 0.1 ppb was reported. Mercury released from solution by $SnCl_2$ has been detected by pneumatoamperometry.[49] In this technique, an electroinactive gas stream containing the electroactive analyte (in this case, mercury vapor) was passed over the surface of a hydrophobic gas porous electrode. The analyte was electrolyzed at constant potential and the resulting current was proportional to the analyte's concentration. The detection limit for mercury was reported to be 5 ppb.

b. Methods for the Speciation of Mercury Compouds in Water

A variety of methods have been used to identify the chemical forms of mercury in water: filtration, centrifugation, dialysis, electrophoresis, extraction, many types of chromatography, and electrochemical techniques.[114]

Techniques such as ultrafiltration have been used to characterize mercury species by their molecular weights.[114] Anodic stripping voltammetry in conjunction with photoxidation by UV light has been used[115] to differentiate between organic and inorganic mercury. Ion specific electrodes have been used to measure the binding of mercury to organic molecules in water.[116]

As was discussed above, selective reduction of mercury species in solution has been used to measure separately inorganic and total mercury concentrations by CV-AAS, with the organic mercury compounds being calculated by difference.[117]

Thin layer chromatography[34,118] and gas chromatography have been used extensively for the identification and quantitation of organomercury compounds in water. Thin layer chromatography has the advantage of needing no elaborate instrumentation. However, the sensitivity is only about 2 μg Hg; therefore, preconcentration of water samples is required.[114] Organomercury compounds must be converted to their chlorides or dithizonates to be separated.

Gas chromatography was the most commonly used method for measurement of organic mercury compounds. The most widely accepted methods were based on the procedure developed by Westoo.[83] The procedure involved acidification of the sample with HCl to form RHgCl compounds (R = alkyl), extraction of the compounds into benzene, partitioning to an aqueous solution of cysteine or thiosulfate, acidification and reextraction into benzene. The RHgCl compounds were separated by gas chromatography and detected with an electron capture detector. The procedure had a number of disadvantages.[122] The electron capture detector was selective for halides, not mercury; therefore, time-consuming and elaborate clean-up procedures were required to eliminate non-mercury containing halides. Large volumes of high purity acids and solvents were required. Inefficient extraction and loss of mercury during sample transfer can result in negative errors. Some studies have reported[2] breakdown of organomercuric halides on the GC column to RHgR compounds, which are not detected by an electron capture detector. Recoveries from spiked samples were generally on the order of 60-80%.[83,122-124]

An investigation of the efficiency of the acidification and extraction procedure was made in the study reported herein. Mercury concentrations in aqueous and benzene layers were measured by AAS with the quartz "T" atomizer.

5. Goals of This Study

The first goal of this study was to develop an improved

analytical method for the determination of total mercury concentrations in water. Each of the aforementioned techniques had its own advantages and disadvantages, but none was a true direct determination, in which no sample pretreatment or preconcentration was required. A direct determination was very desirable because it would eliminate the positive and negative errors associated with pretreatment steps. The accuracy, speed and simplicity of the analysis would be significantly increased.

The Robinson quartz "T" atomizer was ideally suited to the development of a direct analytical method for the determination of mercury in water. The design of the atomizer and the atomic absorption system allowed purging of the light path with a non-absorbing gas such as nitrogen or argon. This permitted the use of the more sensitive 184.9 nm resonance line and, it was hoped, would eliminate the need for preconcentration of mercury from water. The contact time and contact area between the sample and the carbon bed were much greater than those in a conventional graphite furnace. This was expected to result in efficient atomization of all mercury species present in the sample. The efficient atomization would also greatly reduce molecular absorption from unatomized matrix components. All of the sample which entered the atomizer was constrained to exit through the light path. These factors were expected to increase the accuracy of the analysis. In order to test this, water samples would also be analyzed by a standard method,[97] cold-vapor atomic absorption spectrometry.

The second goal of this study was to develop a direct method for the speciation of mercury compounds in water. It was clear that current speciation techniques, which required chemical manipulation of the sample and multiple extraction steps, offered many opportunities for error.

A direct method for separation of compounds by volatility was investigated. The quartz "T" atomizer could be fitted with a resistively-heated platinum wire. A sample could be placed on the wire and slowly heated. Compounds were expected to volatilize at characteristic temperatures and be carried by gas flow to the carbon bed, where atomization would take place. An absorption signal would be measured every time a mercury-containing compound passed through the light path. The appearance temperature of the absorption signal would serve to identify the compound. The use of platinum loop and quartz "T" atomizer in the speciation of aqueous mercury compounds is discussed in Part II of this dissertation. For comparison purposes, the speciation of mercury compounds in water was also investigated by a standard procedure, extraction of alkylmercury compounds into benzene. The extraction study is discussed in this chapter.

B. EXPERIMENTAL

1. Equipment

The major components of the single beam atomic absorption spectrometer used in this research are described below. Some components were designed in this laboratory, while others were

units taken from commercial equipment. The spectrometer was first assembled and used by a former member of this research group, Dr. D. K. Wolcott.[125] A schematic diagram of the complete system is presented in Figure 1. A list of all components is provided.

a. Major Components of the Quartz "T" Atomic Absorption System

i. Light sources: Barnes Demountable Hollow Cathode Lamp System
Beckman Deuterium Lamp

ii. Chopper: Jarrel-Ash Mechanical, from a Model 82-360 AAS

iii. Atomizer: Quartz "T" made of Quartz Scientific Inc. clear fused quartz tubing

iv. Monochromator: Jarrell-Ash Model 82-020, 0.5 m Ebert scanning, with variable slits

v. Detector: Hamamatsu R106-UH photomultiplier

vi. Amplifier: Princeton Applied Research Model 126 lock-in, with Model 184 photometric preamplifier

vii. Recorder: Beckman Model 1005, 10-inch potentiometric strip chart recorder

viii. Detector Power Supply: Hewlett-Packard Model 6515-A, DC, 0-1600V, 0-5 mA

ix. Radiofrequency Generator: Lepel Model T-5-3-KC-E-SW, 5 KHz

x. Cell Vacuum Pump: Thomas Model 107CA20-1

xi. Flowmeters: Matheson Model 7728

xii. Potentiometer: Heliopot Corp. Model T-10-A, 10,000 ohm, ten-turn

xiii. Optics: Amersil Corp. Suprasil grade fused silica lenses with 180.0 nm transmission cutoff

b. Light Sources

i. Barnes Demountable Hollow Cathode Lamp

A commercial Barnes demountable lamp was modified to reduce self-absorption of the mercury resonance lines. Self-absorption was particularly severe in mercury hollow cathode lamps, due to the volatility of mercury. The modifications were designed in this laboratory by R. L. Binder.[76] A hole was drilled through the cathode holder into the cathode. A telfon ring was used to block the space between the anode and the cathode. The filler gas was forced thereby to flow through the interior of the cathode and to exit below the light path. This removed the absorbing sputtered atom cloud from the light path and greatly reduced self-absorption in the lamp. A diagram of the flow-through demountable lamp is given in Figure 2.

A number of different cathode materials were tried. The first was a fused salt cathode. It was prepared by melting

mercuric chloride in a brass cathode cup. The cup was cooled and a hole was drilled through the center of the fused salt. This type of cathode had been used in the determination of mercury in air with the quartz "T" atomizer.[64] The emission spectrum from this cathode is shown in Figure 4. As can be seen, the mercury 184.9 nm resonance line was superimposed on a broad emission background. This was undesirable because it would result in loss of sensitivity. Free atoms are known[63] to absorb radiation only over a narrow range of about 0.002 nm. The spectral bandpass of the monochromator used in this study was 0.2 nm at a slit setting of 100 micrometers. Therefore, a large portion of the radiation falling on the detector was unabsorbable broad band radiation. Absorption of the narrow atomic line would cause only a small decrease in the signal reaching the detector. This would result in poorer sensitivity than if all of the radiation reaching the detector was able to be absorbed. The broad-band emission was probably due to chloride, which introduced the possibility of a positive error if chloride in a sample (urine, blood, water) absorbed this background emission and was interpreted as mercury. Background correction using a deuterium lamp is based on the assumption that atomic absorption measurements are made using a very narrow line source.

Other salts were tried but none were suitable. Hg_2Cl_2, $HgSO_4$ and $HgNO_3$ sublimed and did not fuse, while red HgO decomposed to Hg and reformed black HgO on cooling. The oxide was

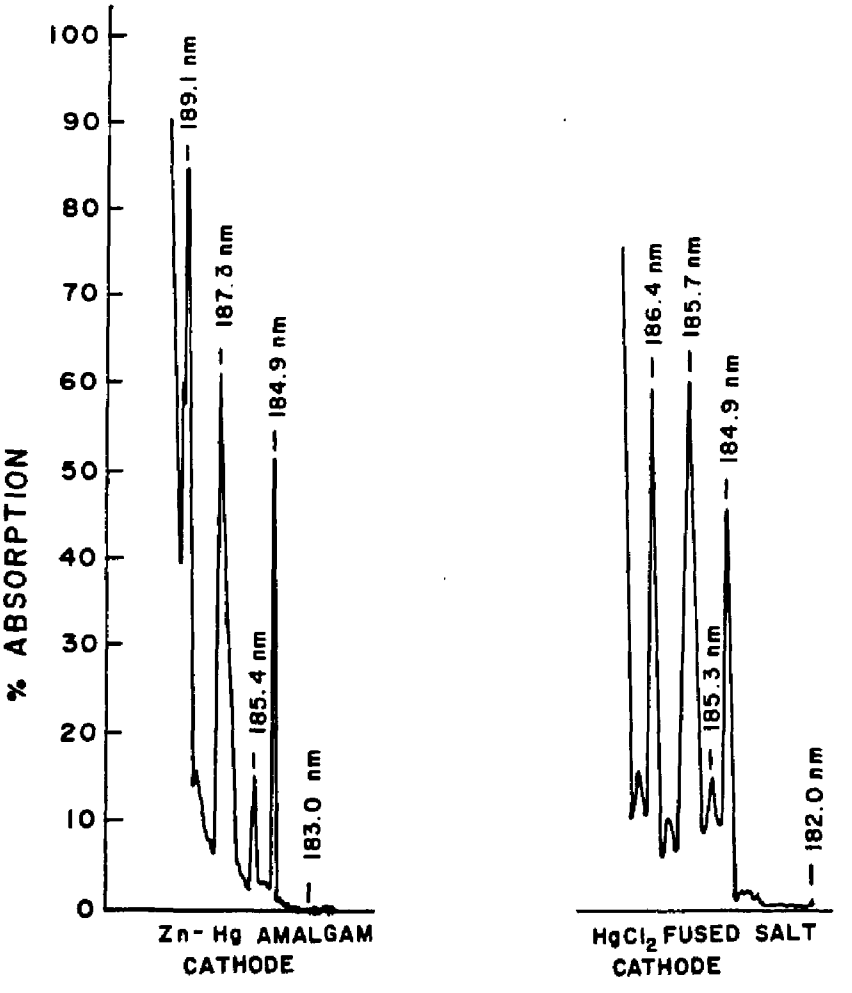


FIGURE 4: EMISSION SPECTRA OF A Zn-Hg AMALGAM CATHODE AND A $HgCl_2$ FUSED SALT CATHODE. THE DEMOUNTABLE HOLLOW CATHODE LAMP WAS USED. THE REGION FROM 182-190 nm IS SHOWN. IN BOTH CASES, THE 184.9 nm Hg RESONANCE LINE WAS SUPERIMPOSED ON BROAD BACKGROUND EMISSION.

deposited as a fine powder and would not adhere to the sides of the cathode cup. An interesting observation was made with regard to the $HgCl_2$ fused salt cathodes prepared in freshly machined brass cups and previously used brass cups. Those prepared in the used brass cathode were white when fused and stayed white; those prepared in new brass cups were white when freshly made, but turned light green overnight and gave reduced intensity at the 184.9 nm line. It is possible that the Hg amalgamated with the brass and was replaced in the salt by Cu.

Due to these problems, several different cathodes were prepared by amalgamating mercury with various metals. A Barnes gold cathode was amalgamated by filling it with distilled elemental mercury, allowing it to stand for a few days and pouring out the excess mercury. A silver-tin mercury cathode was prepared from commercial dental amalgam-filling material. The dental amalgam was packed into a brass cathode cup and allowed to set. A hole was drilled through the center of the amalgam to make the cathode. Squares of copper and zinc foil (1.5 cm long) were amalgamated by soaking in elemental mercury for a few days. The amalgamated foil was rolled into a hollow cylinder with a diameter slightly less than the inner diameter of the brass cathode cup. The foil was inserted into the cathode cup and pressed against the wall of the cup. Emission spectra for these amalgam cathodes are shown in Figures 4-6. The amalgamated copper foil flow-through cathode gave sharp, intense emission lines at 184.9 nm and 253.7 nm, with

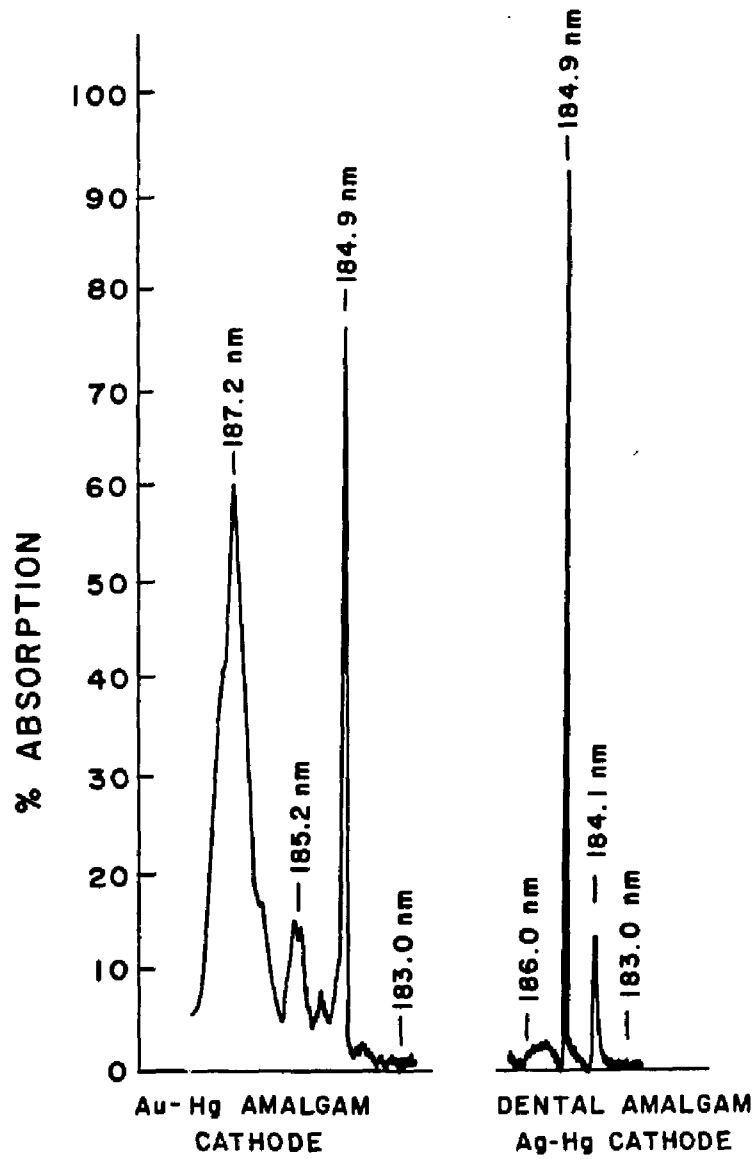


FIGURE 5: EMISSION SPECTRA OF Au-Hg AMALGAM AND Ag-Hg DENTAL AMALGAM CATHODES. THE REGION FROM 182-190 nm IS SHOWN. THE DEMOUNTABLE HOLLOW CATHODE LAMP WAS USED.

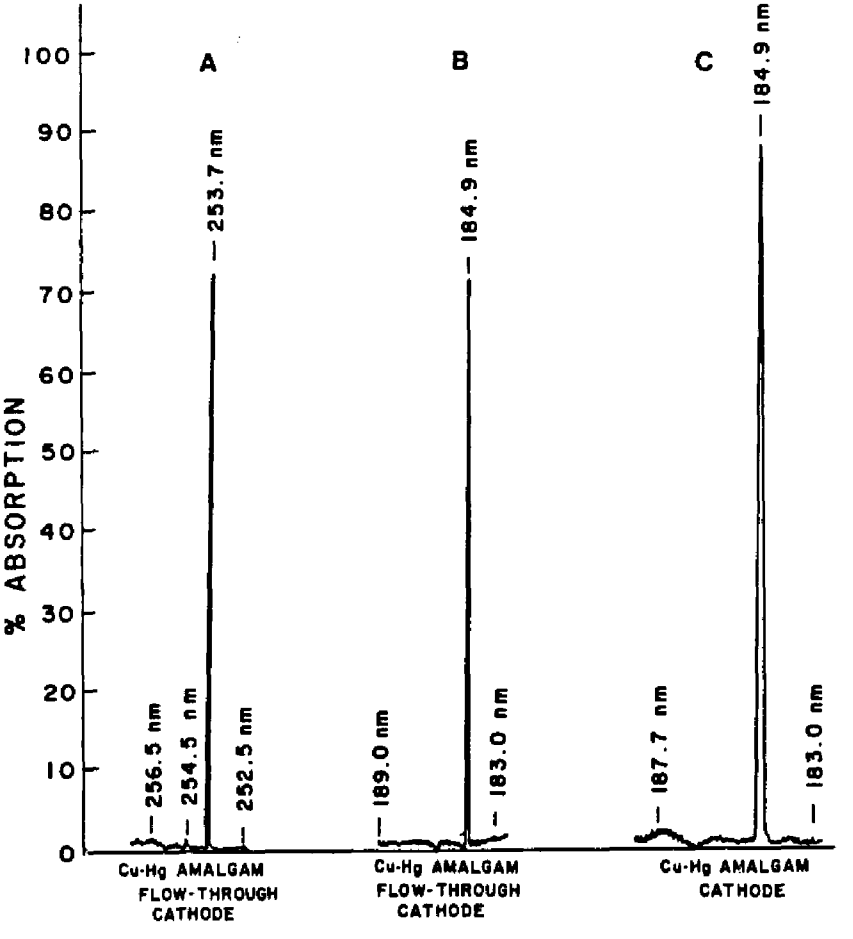


FIGURE 6: EMISSION SPECTRA FROM Cu FOIL-Hg AMALGAM FLOW-THROUGH AND CONVENTIONAL CATHODES. THE FLOW-THROUGH CATHODE GAVE SHARP ATOMIC EMISSION LINES AT 253.7 nm (A) AND 184.9 nm (B) WITH NO BACKGROUND EMISSION OR INTERFERING LINES. THE CONVENTIONAL CATHODE (C) ALSO GAVE ACCEPTABLE EMISSION AT 184.9 nm. THE DEMOUNTABLE HOLLOW CATHODE LAMP WAS USED.

no broad-band emission or interfering atomic lines. It was also easy to prepare and less expensive than silver or gold amalgams. An amalgamated foil cathode lasted for about one month before the mercury was sputtered off and the foil had to be replaced. Amalgamated copper foil flow-through cathodes were used for the work described in this dissertation.

Optimum operating conditions for this cathode were determined by measuring the absorbance of a mercury vapor standard at various currents and filler gas pressures. The combination of helium filler gas at a pressure of 667 Pa and a lamp current of 2 mA generated the greatest absorbance for the standard. These were the operating conditions which were used for analysis of samples.

ii. Beckman Deuterium Lamp

A commercial Beckman deuterium lamp and power supply were used to provide a broad-band source to measure background molecular absorption.

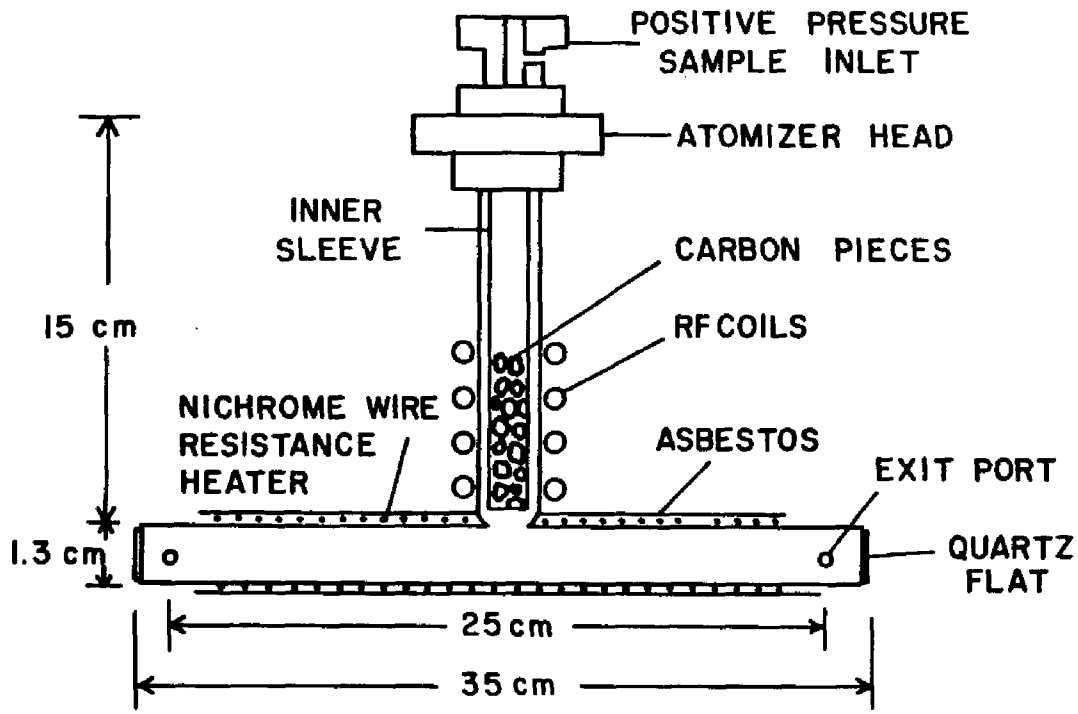
c. Chopper

The chopper created a modulated signal from the light source, which was then directed through the light path of the atomizer. An A.C. amplifier was used to monitor only the interrupted signal created by the chopper. Emission of light by excited atoms in the light path on return to their ground state was D.C. emission and was not detected. The use of a chopper eliminated spectral interference due to emission by components of the sample.

d. Atomizer

The absorption cell was constructed of spectroscopically pure quartz in the shape of a "T". A diagram is given in Figure 7. The crosspiece was 36 cm long, with an I.D. of 1.2 cm. The stem of the "T" was 15.2 cm long, with an I.D. of 2.5 cm. Two quartz disks were fused to the ends of the crosspiece; this served as the optical light path. Two vacuum ports, located approximately 2.5 cm from the ends of the crosspiece, were connected to a vacuum pump which maintained a constant flow through the cell. The crosspiece of the "T" was wound with nichrome wire and several layers of asbestos cord and tape. A current of 7 A was passed through the wire to heat the light path to a temperature of at least 900°C. Heating of the light path was necessary to ensure that atoms entering it from the carbon bed remained in the atomic state.

The vertical stem of the "T" was fitted with a quartz inner sleeve (I.D. = 2.0 cm, O.D. = 2.3 cm). This sleeve was packed with pieces of carbon, approximately 1 cm in length. The carbon pieces were cut from spectroscopically pure rods (0.25 in diameter, Ultracarbon "F" purity, Ultracarbon Corp.). The bottom of the inner sleeve was slightly tapered to hold a porcelain disk. The porcelain disk was 2.0 cm in diameter and was pierced with several concentric rings of holes. It had been the removable bottom of a filtering crucible, but now served the purpose of holding the carbon pieces in the inner sleeve while allowing free air flow



QUARTZ "T" ATOMIZER

FIGURE 7

through the atomizer cell. The inner sleeve prevented devitrification of the absorption cell itself caused by repeated heating of the carbon bed. The inner sleeve was easy to replace when it became divitrified, and was easy to remove for refilling with carbon without disrupting the alignment of the light path.

The carbon bed in the stem of the "T" was heated by radio-frequency (rf) induction and was maintained at 1450°C. An optical pyrometer was used to monitor the temperature of the bed.

Oxygen in the air drawn over the carbon bed was converted to CO at temperatures greater than 900°C. This provided a reducing atmosphere favorable to the formation of free atoms from a sample introduced into the atomizer.

The bed was cleaned before use each day by heating it to 1450°C and waiting until the resonance signal returned to 100% transmission. At this point, it was assumed that no mercury contamination was present on the surface of the carbon.

e. Purging Gases

In order to use the 184.9 nm resonance line, it was necessary to remove as much oxygen from the light path as possible. The monochromator was purged with nitrogen; the atomizer was purged with a mixture of N_2 and O_2. Commercial compressed nitrogen was passed through a scrubbing train of silica gel, resistively-heated copper turnings and activated charcoal to remove oxygen and other impurities. The scrubbed nitrogen was split into two streams, one to the monochromator and one to the

atomizer cell. The nitrogen which entered the atomizer was mixed with a small amount of oxygen (20:1 (V/V) ratio) which had been passed through silica gel and activated charcoal. Some oxygen was needed in the atomizer to insure complete decomposition of biological samples. Purging gas was supplied to the atomizer at 275 mL/min and to the monochromator at 2 L/min.

The first purge gas inlet system for the atomizer consisted of a one-holed rubber stopper with a short piece of glass tubing through it. The glass tubing was attached to the Tygon hose from the scrubbed gas source. The stopper was inserted into the top of the atomizer. The rubber stopper had to be removed to insert a sample. This was undesirable because it allowed ambient air into the atomizer, which absorbed the 184.9 nm line. It also created turbulence inside the atomizer which disturbed the carbon bed and caused carbon dust to fall into the light path. A new inlet system was designed to enable simultaneous introduction of a sample and flushing with scrubbed gas. A brass cap with a "T" intersection, threaded to screw into the top of the atomizer, was made by the departmental machinists (Figure 8). Purge gas was introduced into the stem of the "T" and samples were inserted through the crosspiece.

f. Monochromator and Detector

Light which passed through the atomizer light path was focused onto the entrance slit of the monochromator. The monochromator was modified to allow purging of the interior with

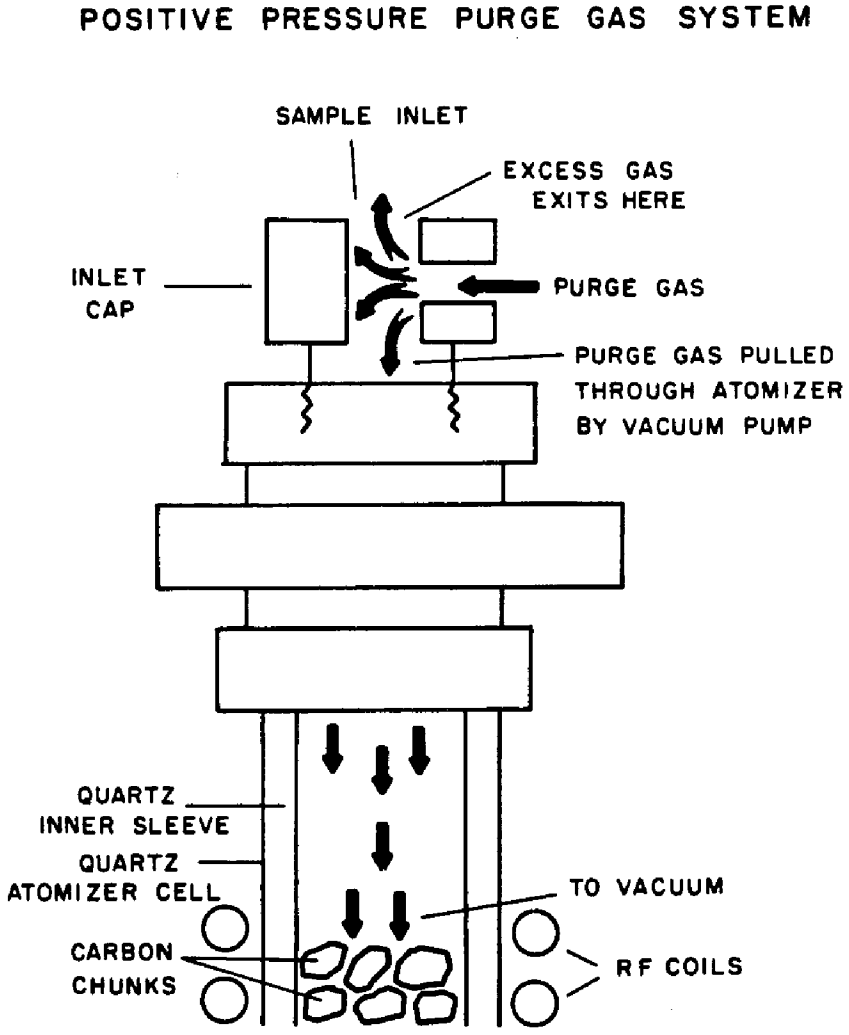


FIGURE 8: THE POSITIVE PRESSURE PURGE GAS SYSTEM EXCLUDED AMBIENT AIR FROM THE ATOMIZER. NO SAMPLE WAS LOST THROUGH THE TOP OF THE ATOMIZER.

scrubbed nitrogen. A hole was tapped in the monochromator housing and a valve with standard 1/4 in. Swagelok fittings was inserted. The valve was connected to the scrubbed nitrogen line. The monochromator contained a diffraction grating (112 lines/cm) which separated the wavelength of interest and focused it onto the photomultiplier. The photomultiplier tube used in this study was a Hamamatsu R106-UH. This tube had a response curve which extended down to 180 nm and was designed for maximum sensitivity in the far ultraviolet region. The signal from the photomultiplier tube (P.M.T.) was amplified using a phase-sensitive A.C. amplifier and recorded on a strip-chart recorder.

g. Lenses and Optical System

Two 3.0 cm diameter, 10 cm focal length, quartz plano-convex lenses were used to maximize the radiant energy available from the system. One lens was situated such that the radiation from the light source was collected and directed along the light path of the quartz "T". The second lens focused radiation emerging from the light path of the quartz "T" onto the monochromator slit.

The radiation sources, lenses, chopper, quartz "T", and monochromator were mounted on a triangular 3-meter optical rail to permit easy and reproducible optical alignment.

h. Other Equipment

i. Eppendorf 5 µL autopipette

ii. Hamilton microliter syringe, No. 701, 10 µL

iii. Drummond 1-5 µL microdispenser

iv. Whatman 41 ashless filter paper

v. Ultracarbon "Graphoil" pyrolytic graphite-coated graphite, .0125 cm thickness

vi. Ultracarbon Ultra "F" purity carbon rods, 0.625 cm diameter

vii. 6 mm paper punch

viii. Optical pyrometer, Leeds and Northrup Model 8632-C, 750°-3500°C range

ix. Hamilton "Gastight" gas syringe #1001, 1 mL capacity

x. Precision Sampling Corporation "Pressure Lok" gas syringe, 10 mL capacity

xi. Stainless steel tweezers

xii. Finnpipette, 1-5 µL autopipette

2. Chemicals and Reagents

All chemicals used in these studies were ACS Reagent Grade whenever possible. Those which were not will be so noted. Deionized distilled water was used for the preparation of all reagents, standards, and dilutions.

a. Distilled elemental mercury

b. Stock 1000 ppm Hg (inorganic) standard. Prepared by dissolution of 1.3540 g $HgCl_2$ (MCB, Inc.) and 1.5 mL conc HNO_3 (MCB, Inc.) and dilution to 1 L with deionized distilled water

c. Stock 1000 ppm Hg (organic) standard. Prepared by dissolution of 0.1251 g CH_3HgCl (Alfa Inorganics) and dilution to 100 mL with benzene (Mallinckrodt)

d. 10% (V/V) HNO_3

e. Benzene (Mallinckrodt)

f. 1% cysteine acetate. Prepared by dissolution of 1 g L-cysteine monohydrochloride (General Biochemicals, not reagent grade), 0.8 g sodium acetate (Allied Chemical) and 12.5 g sodium sulfate (Allied Chemical) in 100 mL deionized distilled water. The solution was washed 3x with 10 mL portions of benzene.

g. Concentrated HCl (MCB, Inc.)

h. 1000 ppm Hg stock standard, commercially prepared (Kemron Environmental Services)

i. Potassium permanganate solution. Prepared by dissolution of 50 g $KMnO_4$ (Mallinckrodt) and dilution to 1 L with deionized distilled water

j. Potassium persulfate solution. Prepared by dissolution of 50 g $K_2S_2O_8$ (Baker) and dilution to 1 L with deionized distilled water.

k. Sodium chloride-hydroxylamine sulfate solution. Prepared by dissolution of 120 g NaCl (MCB, Inc.) and 120 g $(NH_2OH)_2 \cdot H_2SO_4$ (Mallinckrodt) and dilution to 1 L with deionized distilled water

l. Stannous chloride solution. Prepared by dissolution of 100 g $SnCl_2$ (Baker) and 12.5 mL conc HCl and dilution to 1 L with deionized distilled water.

m. Concentrated H_2SO_4 (MCB, Inc.)

n. Deionized distilled water. Prepared by passing in-house distilled water through a mixed-bed ion exchange column (Ionexchanger model 2, Illinois Water Treatment Co.).

3. Procedures for the Direct Determination of Mercury in Water

a. Operating Parameters

Typical operating parameters were as follows:

i. Hollow cathode lamp: 2 mA current, He filler gas

ii. Carbon bed temperature: 1450°C

iii. Atomizer purge gas: Purge gas was supplied to the atomizer at 275 mL/min, slightly faster than the usually cell pumping rate of 250 mL/min. This provided a positive pressure system and prevented the entrance of ambient air into the atomizer. (Figure 8.)

iv. Light path temperature: 900°C

v. Slit width: 100 μm (at 184.9 nm resonance line); 25 μm (at 253.7 nm resonance line)

vi. Wavelength: 184.9 nm; 253.7 nm

vii. Monochromator purge gas: scrubbed N_2, 2 L/min.

viii. P.M.T. voltage: 500V

ix. Cell pumping rate: 250 mL/min

x. Amplifier: ACVM mode, 200 uV sensitivity, 100 ms time constant

xi. Recorder: 100 mV, linear mode

b. Sample Collection

Water samples were collected from several sources in the Chemistry building. Deionized distilled water from this laboratory and from 2 other laboratories was analyzed. Tap water from several faucets in this laboratory was analyzed. All in-house water samples were collected immediately before analysis in nitric acid-cleaned Pyrex test tubes.

Tap water samples were collected from a number of Baton Rouge private homes and apartments. Tap water samples from El Dorado, Arkansas, lake water from Lake Maurepas, Louisiana and from the Gulf of Mexico were collected. These samples were collected in nitric acid-cleaned 250 mL polyethylene bottles with screw caps. No preservative was added to avoid contamination of the samples. Samples were analyzed as soon as possible after collection. In most cases, this was within 24 hours of collection.

c. Development of a Technique for Liquid Sample Introduction

i. Direct Injection with a Micropipette

Sample introduction into the atomizer was attempted by direct injection of 5 μL aliquots of water with an

Eppendorf or Finnpipette micropipette. The sample was injected onto a hot (1450°C) carbon bed. At 184.9 nm, 5 µL aliquots of deionized distilled water exhibited background absorption signals of about 90% absorption, so a smaller aliquot was required. This ruled out the use of the micropipette at 184.9 nm, because a 1 µL aliquot would not fall off the end of the pipette tip onto the carbon bed. Injection of a 5 µL aliquot of water generally resulted in a 10-15% resonance absorption signal and 0-5% background absorption signal at 253.7 nm. Although these signals were of reasonable intensity, the method suffered from poor reproducibility. Absorption signals varied in shape from sharp spikes to broad tailing peaks. Injection of 5 µL aliquots often caused visible disruption of the carbon bed surface, with carbon dust being dislodged into the air above the bed. After some injections, an explosion occurred as the sample burned, which blew hot gases out of the top of the atomizer. These observations indicated that one reason for poor reproducibility was a difference in reaction of the sample at the carbon surface. The lack of reproducibility rendered this method of sample introduction unsuitable.

ii. Filter Paper Disk Method

Small filter paper disks had been used for the introduction of aqueous cadmium standards into the quartz "T" atomizer.[126] Introduction of a 1 µL sample on a filter paper disk had been found to reduce molecular absorption by increasing the contact time and contact area between the sample and the

carbon bed. Samples which were introduced on these disks appeared to burn more evenly than samples which were injected directly into the atomizer.

Filter paper disks were prepared by cutting Whatman #41 ashless filter paper into disks of 6 mm diameter with a paper punch. Disks were cleaned by two 24 hour soakings in 10% HNO_3, using two fresh portions of acid. The disks were then placed in a Buchner funnel, rinsed with copious amounts of deionized distilled water, and partially dried by application of vacuum-suction. The disks were covered by a sheet of filter paper during the drying step. Disks were allowed to dry completely by standing at room temperature in a clean air environment.

One or two microliter aliquots of water were placed on filter paper disks with a Hamilton microliter syringe. This eliminated the problem of drop hang-up which occurred with direct injection, because the syringe tip was touched to the disk and the liquid was drawn out by capillary action. Disks were picked up and dropped into the atomizer with a pair of stainless steel tweezers. The tips of the tweezers were cleaned periodically by being held in a Bunsen burner flame.

Acid-cleaned filter paper disks with no sample on them generated large and widely-varying resonance absorption signals of 40-60% absorption at 184.9 nm. The fluctuations in absorption intensity were probably due to inconsistent cleaning of the disks by the method used. Attempts were made to volatilize mercury from

the filter paper disks by heating acid-cleaned disks in the atomizer. A layer of disks was placed on top of a few pieces of carbon in the inner sleeve. The rf generator was not turned on; disks were heated by air from the heated light path until they were slightly charred. This helped to reduce both the resonance and background absorption signals. The charring of the disks was not uniform and the absorption signals were still erratic. The size of the disks was decreased by using a 3 mm-diameter paper punch. Significant absorption of both the resonance and deuterium signals was still observed and the reproducibility was still poor. For example, heat and acid-cleaned 3 mm filter paper disks generated an average resonance absorption signal of 30.7 $\pm$ 7.9% absorption (mean $\pm$ σ) and a background absorption signal of 12.2 $\pm$ 2.0% absorption, while uncleaned filter paper disks generated average resonance and background absorption signals of 375. $\pm$ 14.9% and 18.4 $\pm$ 7.5%, respectively. Acid and heat cleaning therefore resulted in a 22% decrease in resonance absorption and a 35% decrease in background absorption compared to uncleaned disks. A comparison of absorption signals from cleaned and uncleaned filter paper disks is presented in Table 2.

A 30% absorption signal from a blank filter paper disk was felt to be too high to allow quantitative analysis, so other methods of sample introduction were investigated.

iii. Carbon Disk Method

Introduction of liquid samples into the atomizer

on small disks was still an attractive idea, so a material was sought which would give smaller absorption signals than filter paper. Carbon disks had been tried by previous researchers in this group[125,126] without too much success. They had used slices of 0.25 cm-diameter spectroscopic carbon electrodes (Ultracarbon Corp.); the slices were about 0.1-0.2 cm thick. When dropped onto the carbon bed, the disks dislodged carbon dust into the light path. On repeated heating, the disks became porous; sample soaked into the disk and took a long time to diffuse out. This resulted in broad tailing peaks and decreased sensitivity. Disks built up rapidly on the top of the carbon bed. This lowered the temperature of the surface, since the disks did not couple with the rf field, and forced frequent shut-downs to remove accumulated disks.

Mr. Carl Leistner, of the Ultracarbon Corp., provided a new material for use in carbon disks. The material was "Graphoil," a thin pressed graphite sheet. The sheets were 0.0125 cm thick and were coated with pyrolytic graphite. Disks of 6 mm diameter were cut from the "Graphoil" sheets with a paper-punch. The carbon disks were cleaned by placing them on the hot carbon bed (1450°C) for 30 minutes, or until the absorption of the mercury resonance line returned to zero. The carbon disks were cooled to room temperature under scrubbed nitrogen, removed from the inner sleeve and stored in capped polyethylene vials. The disks were cleaned approximately 1 hour prior to use, as it was observed that they

would absorb mercury from the laboratory air on standing. Resonance absorption increased from 0 to 27% absorption and background absorption increased from 0 to 5% over a 24 hour period. A plot of the absorption signal with time for cleaned carbon disks is given in Figure 9.

Aliquots of 1 or 2 microliters were placed on carbon disks with a Hamilton microliter syringe. The disks were dropped onto the hot carbon bed with cleaned stainless steel tweezers. Since these disks were much lighter and thinner than those cut from the electrodes, the problems of carbon dust dislodgement and broad absorption peaks did not occur. After several heating cycles, the Graphoil disks did become porous, but they were so thin that samples readily diffused out. Approximately 50 injections could be made before the temperature of the carbon bed dropped significantly. No contribution to the absorption signal at either 184.9 nm or 253.7 nm was made by the Graphoil disks. Introduction of liquid samples on carbon disks proved to be a very successful technique; it was used extensively in the studies reported in this dissertation.

iv. Direct Injection With the Drummond Microdispenser

Another technique which proved to be successful for the injection of liquid samples was direct injection with a modified Drummond microdispenser. The microdispenser was capable of delivering 1-5 μL aliquots. It was unsatisfactory as originally delivered, due to excessive drop hang-up on the end of the

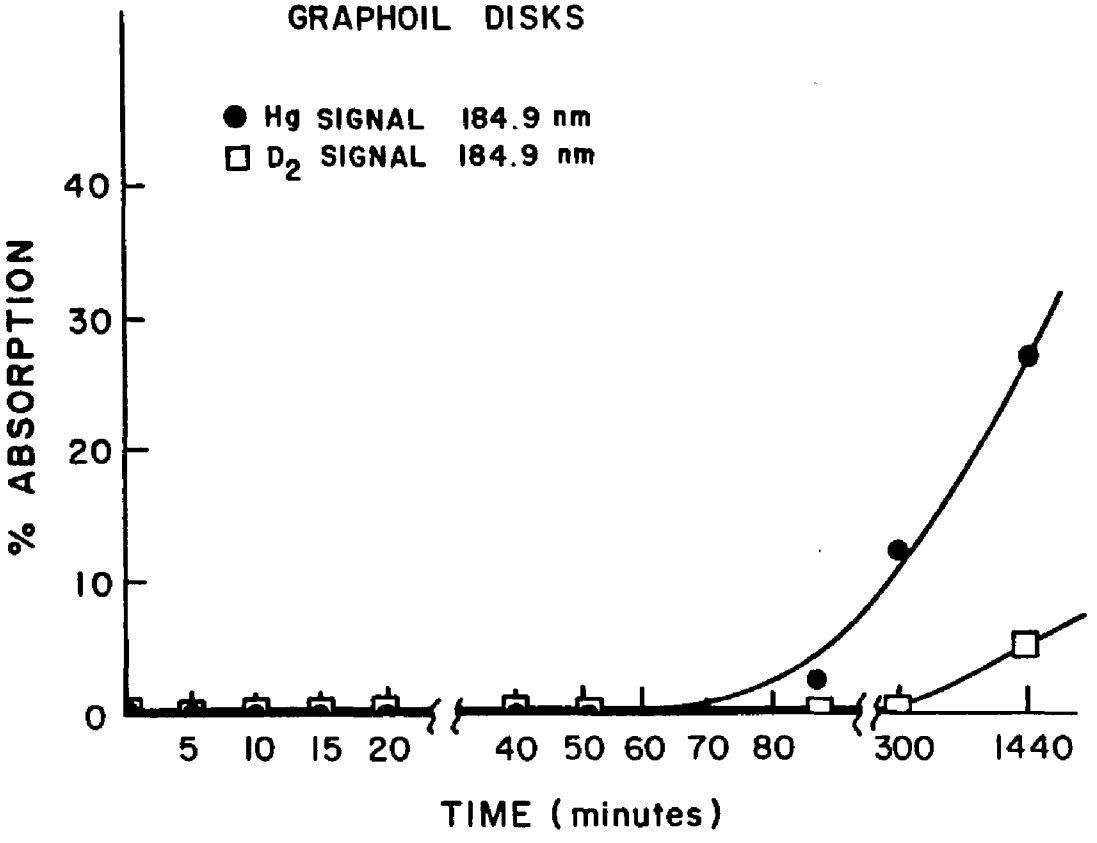


FIGURE 9:ABSORPTION OF MERCURY FROM AIR BY CARBON DISKS. HEAT-CLEANED 6mm "GRAPHOIL" DISKS ABSORBED MERCURY ON EXPOSURE TO AMBIENT AIR. MEASURABLE ABSORPTION OCCURRED AFTER ONE HOUR AND INCREASED OVER THE 24 HOUR OBSERVATION PERIOD. ABSORBED MERCURY COULD BE REMOVED BY HEATING THE CARBON DISKS.

glass barrel. The dispenser was modified by Dr. D. K. Wolcott,[125] formerly of this research group. By drawing a fine capillary tip on the end of the disposable glass barrel and transferring the travel-limiting sleeve from inside the dispenser body to the plunger, the microdispenser was converted to an air-displacement device. (Figure 10.) In the modified device, an excess volume of air was retained between the plunger and the sample. When the plunger was depressed, the excess air assured that the entire volume of liquid was ejected from the barrel.

One or two microliter aliquots were injected directly onto the hot carbon bed. This direct injection technique eliminated the few problems that were encounted with carbon disk injection, such as a build-up of disks on the surface of the carbon bed. The Drummond microdispenser worked well for the injection of most liquid samples which were examined in the studies reported here. The device was unsuitable for the injection of blood and serum, due to their high viscosity. In addition, the plunger was corroded badly by the strong HCl solutions which were injected during the CH_3HgCl extraction studies.

v. Other Methods of Sample Introduction

Numerous other methods for introduction of liquid samples into the atomizer were investigated. These will be discussed only briefly, since they were not successful for determination of mercury.

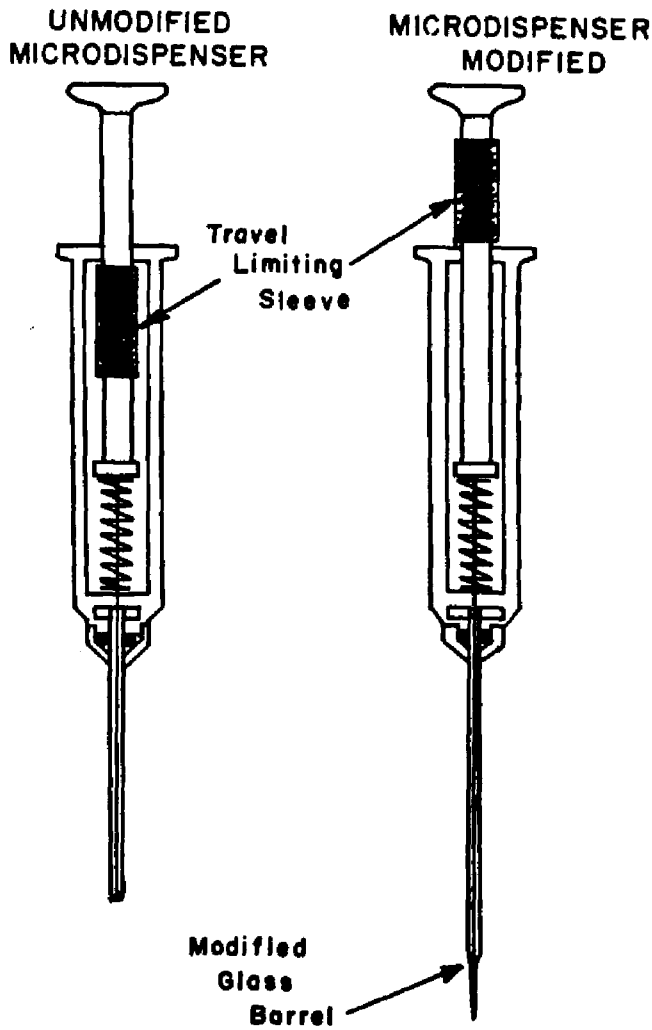


FIGURE 10: THE DRUMMOND MICRODISPENSER WAS MODIFIED BY DRAWING A FINE CAPILLARY TIP ON THE GLASS BARREL AND MOVING THE TRAVEL-LIMITING SLEEVE TO THE PLUNGER. THIS ENSURED COMPLETE AND REPRODUCIBLE EJECTION OF SAMPLE.

Liquid sample introduction had been performed[125] by injection of liquid into short segments of polyethylene tubing. The tubing segments were dropped onto the hot carbon bed with a pair of tweezers. In the current study, 1 cm segments of polyethylene capillary tubing were analyzed. Unfortunately, background signals of 70% absorption were generated by the tubing segments at 253.7 nm. Smaller pieces of tubing could not be used because they would not accommodate 1 µL of fluid.

A solid glass rod was used as a volatilization stage for liquid samples. An aliquot of sample (1-5 µL) was placed on the end of the rod, which then was inserted into the atomizer. The end of the rod was positioned about 2.5 cm above the surface of the carbon bed. It was thought that the temperature would be sufficiently high to vaporize all mercury-containing species in the sample. Absorption traces generated by samples on the glass rod were very broad, due to the slow rate of heating. This decreased the sensitivity, but aqueous mercury standards were successfully analyzed by this technique. Some matrices were not completely volatilized from the glass; urine samples left a white sal-like deposit and blood samples left a charred organic residue. Therefore, this technique was not used for quantitative studies for fear that compounds were not completely volatilized. The glass rod was used with some success in speciation studies of mercury compounds, discussed in Part II of this dissertation.

A modification of the glass rod was developed in an attempt to improve the volatilization efficiency. If a carbon segment, identical to those composing the carbon bed, was lowered into the rf field, it would couple with the field and become hot. An ideal sample introduction technique would be to place a sample on a carbon segment, lower it into the rf field, allow the sample to atomize and retrieve the carbon piece. A glass rod shaped like a ladle was designed to hold a carbon segment at one end. Unfortuntely, the dimensions of the atomizer inlet were only large enough to permit a 6 mm O.D. glass rod to be used. The 0.25 cm diameter carbon chunk had to be shaved down to 5 mm to fit in the rod. The carbon segment was then too small to couple effectively with the rf field and so did not heat well. To use a larger carbon piece, it would have been necessary to redesign the brass atomizer top. This step was not thought to be worthwhile, since the carbon disk method and Drummond microdispenser method were perfectly adequate for the investigations to be undertaken.

d. Calibration

Two approaches to calibration were used for most of the studies reported in this dissertation. The first was injection of air saturated with mercury vapor; the second was injection of aqueous mercury standards prepared from mercuric chloride. A third calibration procedure was used in the methylmercury extraction study; this involved injection of solutions of methylmercury chloride in benzene.

1. Mercury Vapor Standard

If mercury metal at a known temperature and pressure was introduced into a closed container, the air above the metal became saturated with mercury vapor. Since mercury vapor was monatomic, the ideal gas law was used to calculate the weight of mercury contained in a given volume of air at a given temperature. The ideal gas law was expressed as

$$\frac{w}{V} = \frac{P(at.wt.)}{RT} \qquad (7)$$

where w = weight of element

V = volume

P = pressure

$at.wt.$ = atomic weight of element

R = gas constant

T = temperature

The vapor pressure of mercury at various temperatures was accurately known.[127] For example, at 20°C, the vapor pressure of mercury was 159.99×10^{-3} Pa. Substitution into the above equation yields

$$\frac{w}{V} = \frac{(159.99 \times 10^{-3}\,Pa)(200.6 \times 10^{9}\,ng/mole)}{(8.314 \times 10^{6}\,\frac{cm^3 \cdot Pa}{K \cdot mole})(293K)} = 13.17\,\frac{ng}{cm^3}$$

Thus, 1 cm^3 of air saturated with mercury vapor at 20°C contained 13.17 ng Hg. Similar calculations at various temperatures yielded the values shown in Figure 11.

Distilled elemental mercury was placed in 25 mL glass bottles. The bottles were capped with rubber septa and sealed with aluminum seals. A hole in the center of the aluminum cap allowed insertion of a needle through the septum into the headspace over the mercury pool. Gas-tight syringes were used to withdraw aliquots of air saturated with mercury vapor. Three bottles were maintained so that vapor could be withdrawn at frequent intervals with confidence that equilibrium conditions were established. Aliquots of air saturated with mercury vapor were injected through the brass inlet cap directly into the atomizer.

Typical calibration curves obtained with mercury vapor injections are shown in Figures 12 and 13. Calibration curves were linear up to 3 ng Hg (about 0.3 mL of air saturated with mercury vapor) at 184.9 nm and up to 20 ng Hg (about 2 mL of air saturated with mercury vapor) at 253.7 nm. The precision of the mercury vapor injection technique was determined by making 20 injections of a 0.5 mL aliquot (about 1 ng Hg). In all cases, straight lines were fitted to the calibration data by the method of least squares.

ii. Aqueous Mercuric Chloride Standard

Aqueous 1000 ppm Hg(II) standard was diluted daily to prepare working standards. Standards in the range of 0.01-3 ppm were prepared for analysis at 184.9 nm, and in the

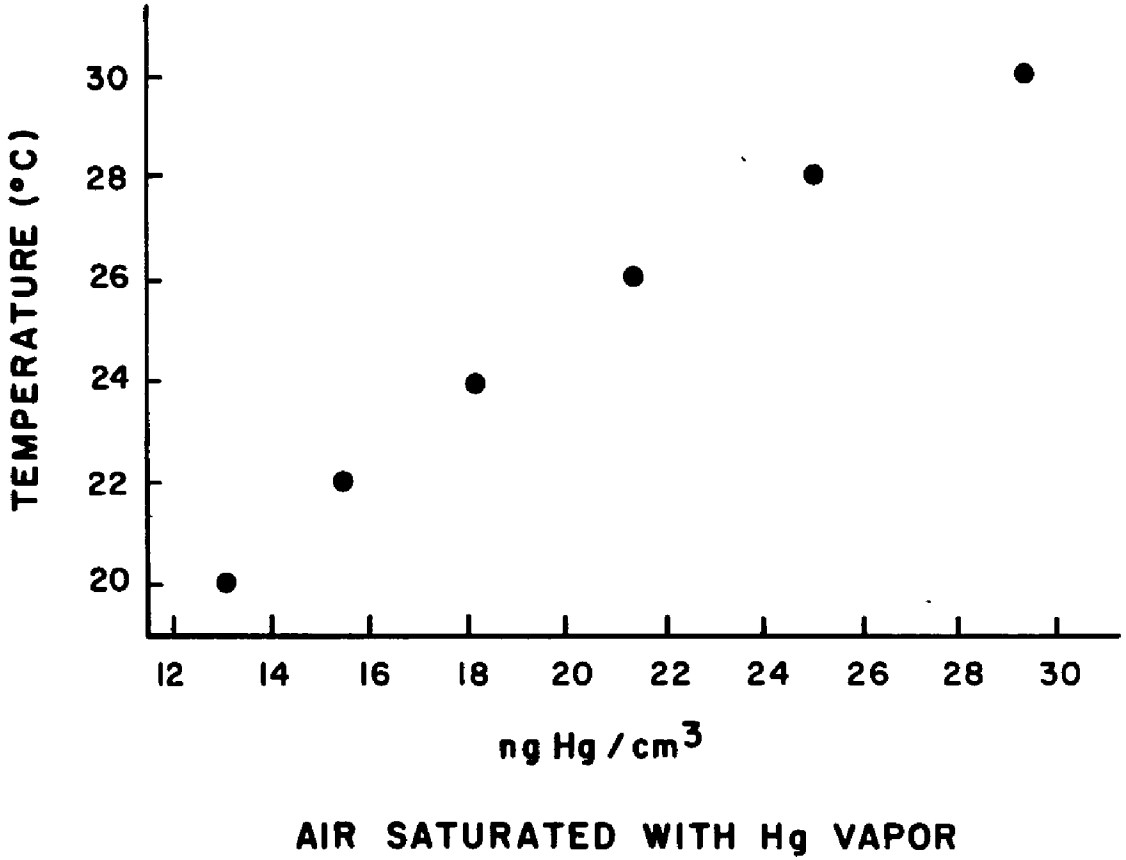


FIGURE 11: USE OF THE IDEAL GAS LAW AND VAPOR PRESSURE DATA ALLOWED CALCULATION OF THE AMOUNT OF Hg IN AIR SATURATED WITH MERCURY VAPOR AT VARIOUS TEMPERATURES.

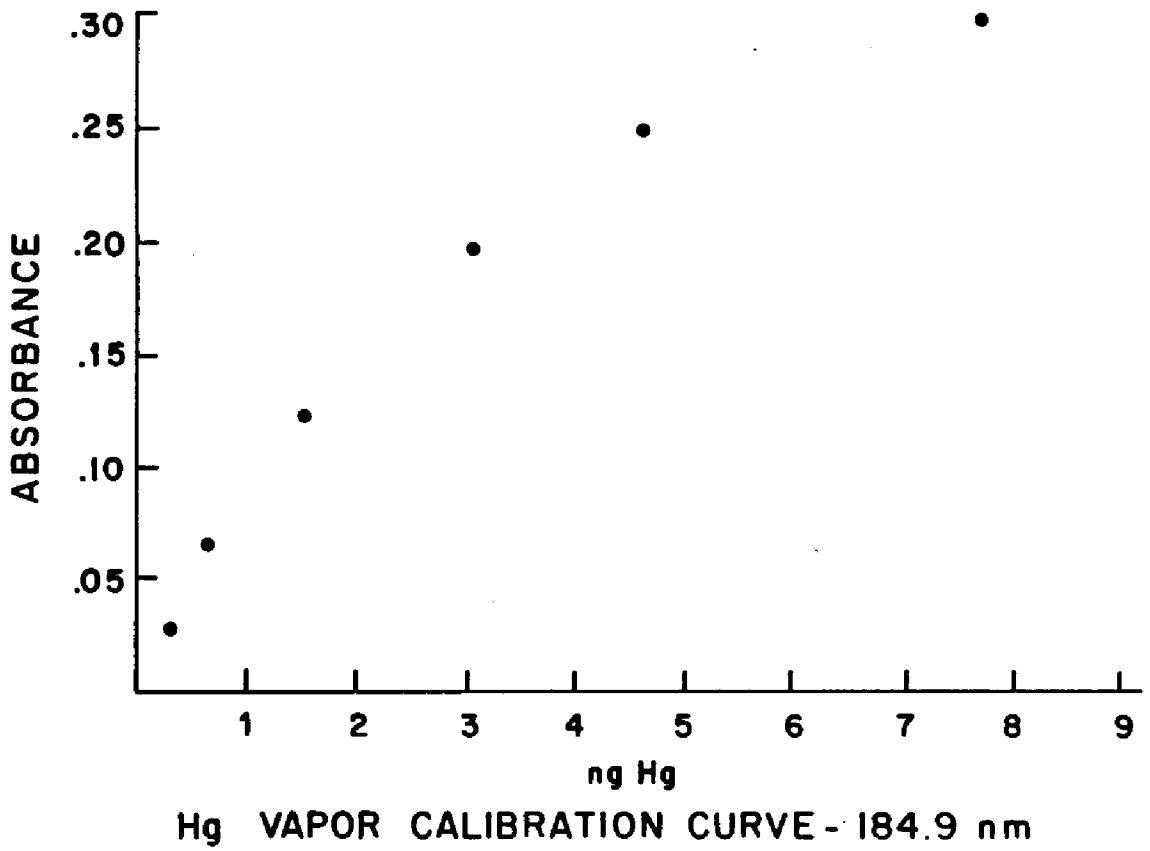


FIGURE 12: CALIBRATION CURVE AT 184.9 nm PREPARED BY INJECTION OF AIR SATURATED WITH Hg VAPOR. THE DEVIATION FROM LINEARITY IS DUE TO SELF-REVERSAL OF THE RESONANCE LINE WITHIN THE LAMP.

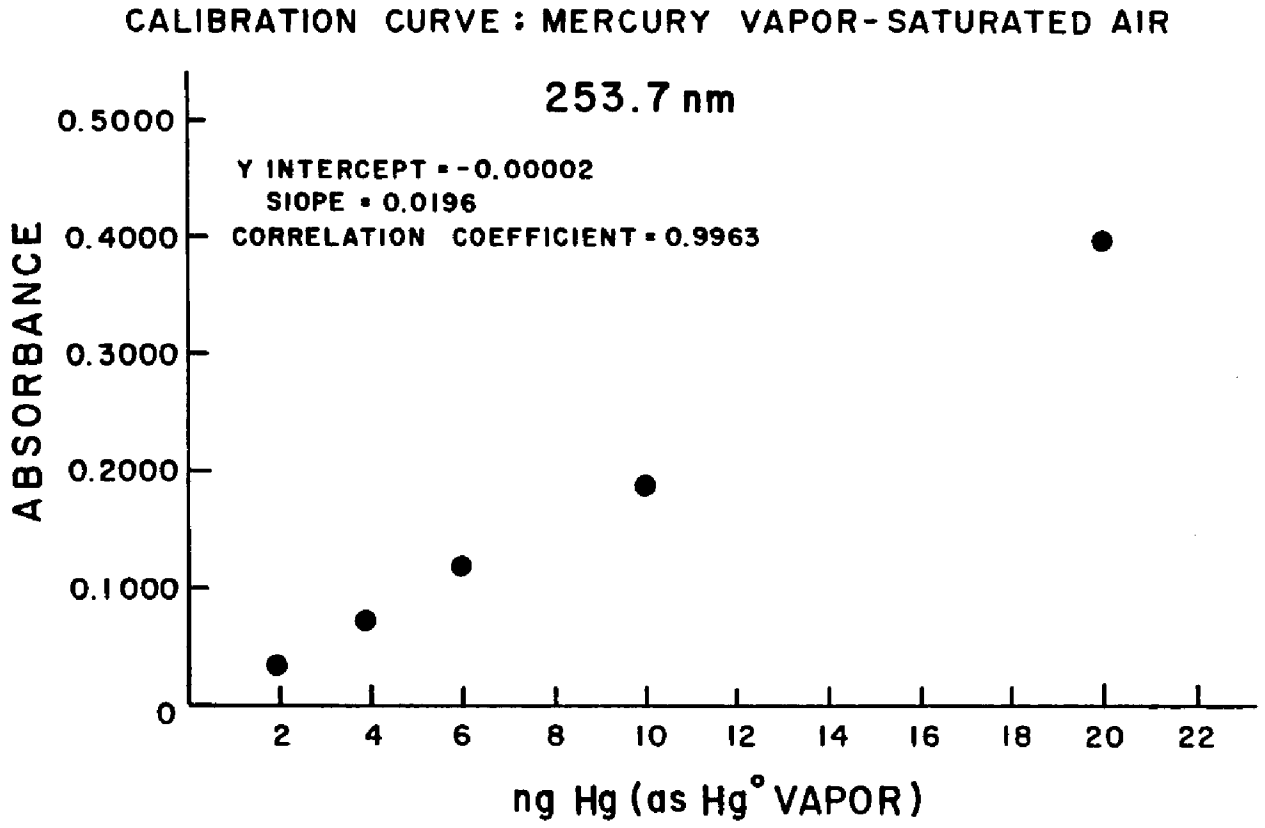


FIGURE 13: TYPICAL CALIBRATION CURVE AT 253.7 nm PREPARED BY INJECTION OF AIR SATURATED WITH Hg VAPOR.

range of 0.1-20 ppm from analysis at 253.7 nm. Distilled deionized water was used to prepare all standards. Calibration curves were prepared by two methods, introduction of 1 µL aliquots on a carbon disk and direct injection of 1 or 2 µL aliquots with the Drummond microdispenser. Typical calibration curves are shown in Figures 14-16.

Precision was determined by analysis of 20 aliquots each of tap water and 2 ppm Hg standard.

iii. Methylmercuric Chloride Standard in Benzene

The 1000 ppm Hg (as CH_3HgCl) in benzene standard was diluted daily to prepare working standards for the CH_3HgCl extraction studies. Standards in the range of 0.1-2.0 ppm were prepared for analysis at 253.7 nm. Calibration curves were prepared by two methods, introduction of 1 µL aliquots on a carbon disk and direct injection of 1 or 2 µL aliquots with the Drummond microdispenser. Pure benzene was analyzed as a blank. No absorption signal was seen for benzene at 253.7 nm, which indicated the superb atomization efficiency of the carbon bed, since the benzene molecule has an absorption maximum at 253 nm. Precision was determined by analysis of 20 aliquots of a 2 ppm Hg standard.

Calibration was checked in two ways: by analysis of a commercially-prepared mercury standard provided by Kemron Environmental Services and by analysis of check samples prepared by an independent party in this laboratory.

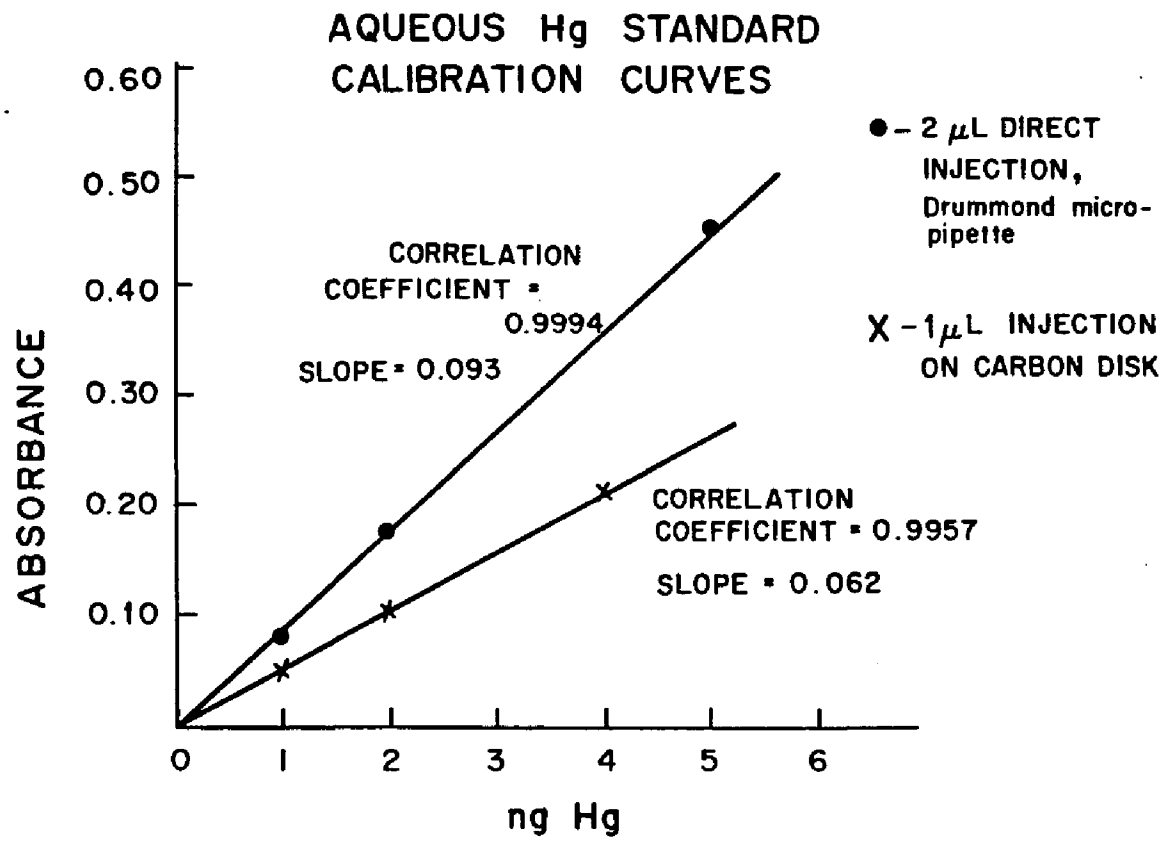


FIGURE 14: CALIBRATION CURVES AT 184.9 nm PREPARED BY INJECTION OF AQUEOUS MERCURY STANDARDS. CURVES OBTAINED BY THE CARBON DISK METHOD AND BY DIRECT INJECTION WITH THE DRUMMOND MICRODISPENSER ARE SHOWN. THE SLOPES OBTAINED BY THE TWO INJECTION TECHNIQUES ARE NOT EQUAL; THEREFORE STANDARDS AND SAMPLES MUST BE INJECTED IN THE SAME MANNER FOR COMPARISON.

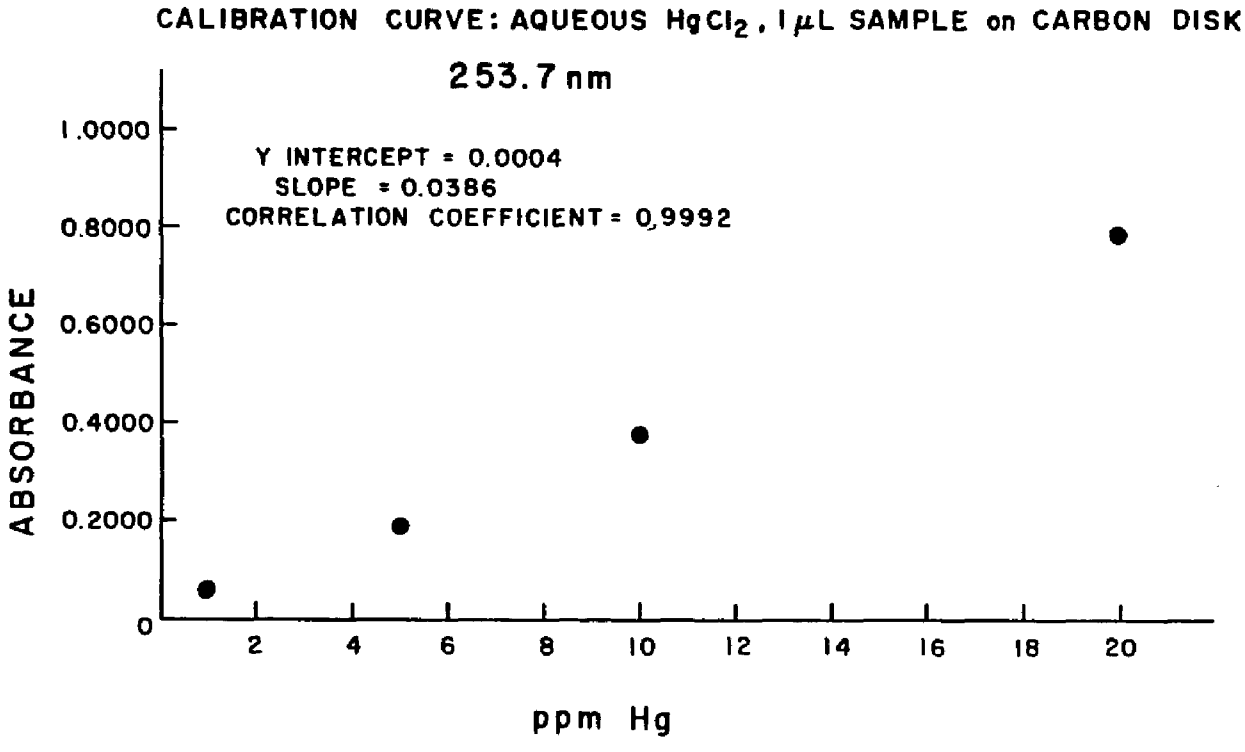


FIGURE 15: TYPICAL CALIBRATION CURVE OBTAINED AT 253.7 nm BY INJECTION OF AQUEOUS STANDARDS ON CARBON DISKS.

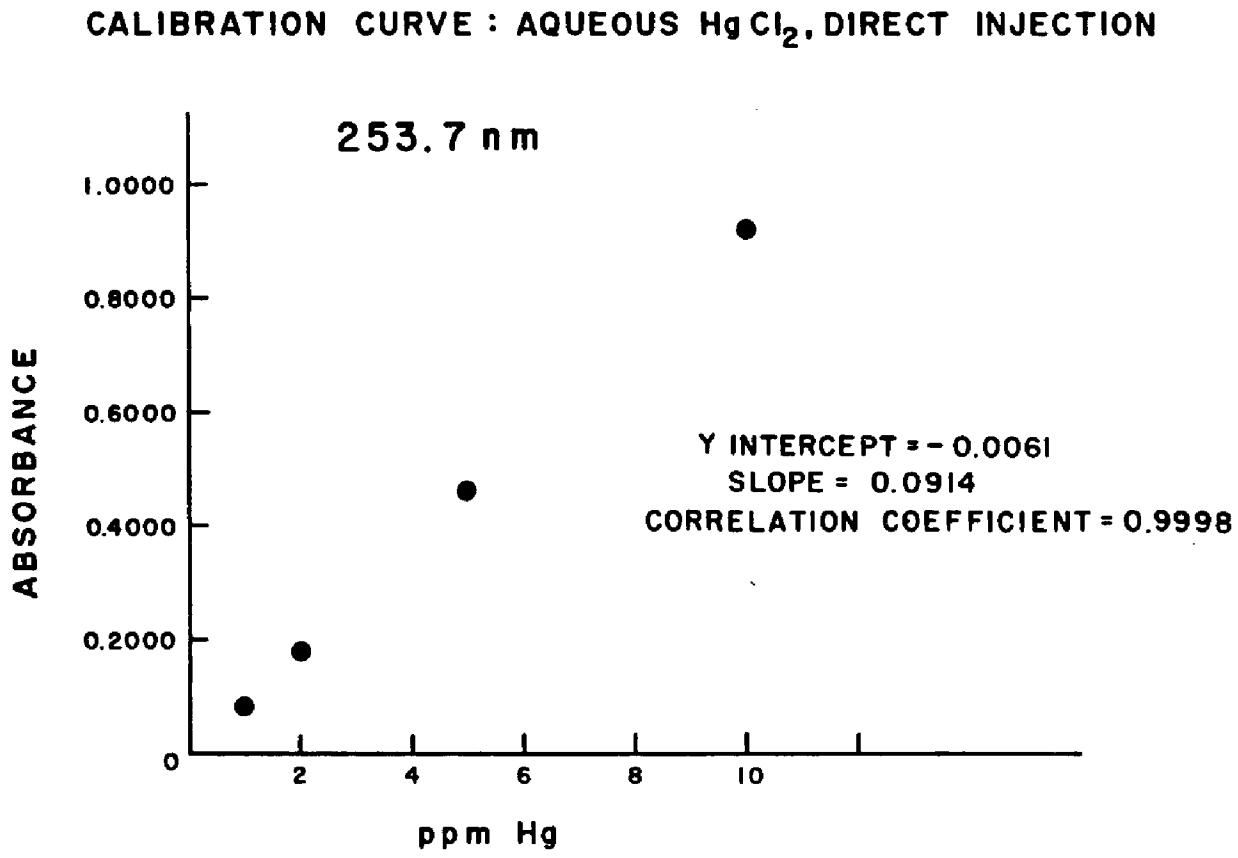


FIGURE 16: TYPICAL CALIBRATION CURVE OBTAINED AT 253.7 nm BY DIRECT INJECTION OF AQUEOUS STANDARDS.

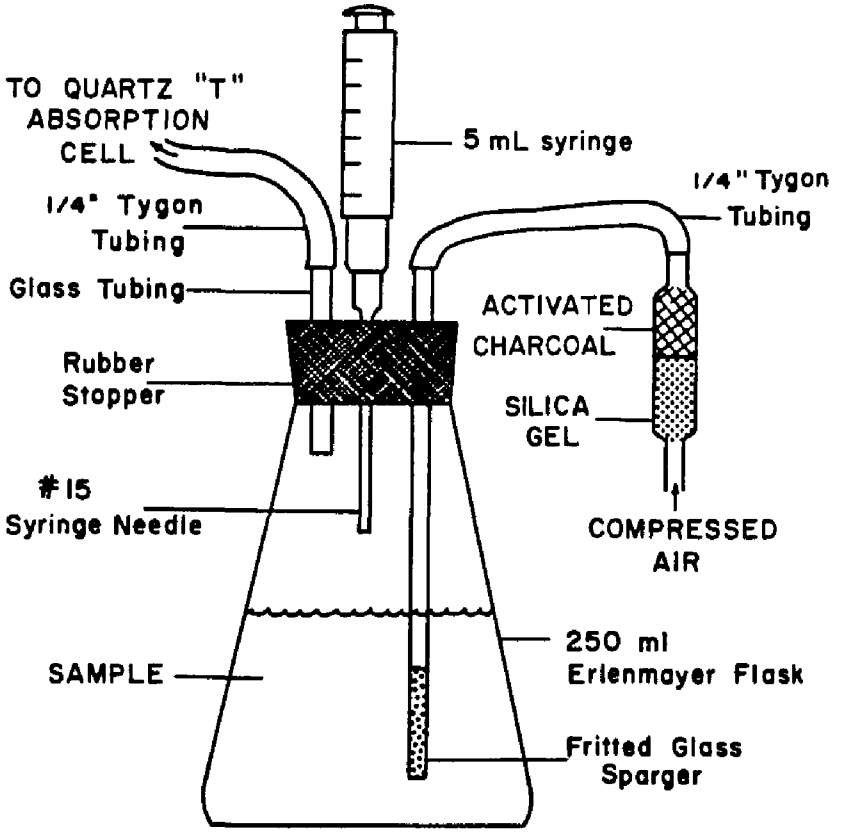
e. Analysis of Mercury in Water by the Cold Vapor-AAS Method

A study was made of the CV-AAS technique for mercury determination using the quartz "T" atomizer as the absorption cell. The inner sleeve and carbon bed were removed from the atomizer. The rf generator was not turned on and the light path was not heated. The top of the atomizer was fitted with a one-holed rubber stopper and was connected to the reaction vessel via a short piece of glass tubing through the stopper and a length of 1/4" Tygon tubing.

The reaction vessel was a 250 mL Erlenmayer flask sealed with a three-holed rubber stopper. A fritted glass sparger through one of the holes was connected to the in-house compressed air. The compressed air was run through scrubbers of silica gel and activated charcoal before entering the reaction flask. The second hold held a short piece of glass tubing to which was attached the Tygon tubing leading to the atomizer cell. The third "hole" was a stainless steel screw-on syringe needle (B-D #5) which had been thrust through the stopper. This served as the inlet for the $SnCl_2$ solution. The solution was taken up in a 5 mL glass syringe (B-D "Multifit") and the syringe was screwed into the hub of the needle. The plunger was depressed to inject the solution and remained depressed until the mercury absorption signal had been recorded. Then the syringe was removed and refilled for the next injection. The sparger, needle and glass tubing were sealed

into the rubber stopper with silicone rubber sealant to avoid leaks. A diagram of the reaction flask is shown in Figure 17.

Analysis was performed exactly as described in Standard Methods for the Examination of Water and Wastewater.[97] The compressed air flow rate was set a 2 L/min. Aliquots (100 mL) of mercury standards, tap water and deionized distilled water were transferred in duplicate to 250 mL Erlenmayer flasks. Each flask received 5 mL conc H_2SO_4, 2.5 mL conc HNO_3 and 15 mL potassium permanganate solution and then was allowed to stand for 15 minutes. Potassium persulfate solution (8 mL) was added and flasks were heated in a water bath at 95°C for 2 hours. Flasks were then cooled to room temperature and 6 mL sodium chloride-hydroxylamine solution was added to reduce excess permanganate. Each flask was then treated individually. The stopper containing the aeration apparatus was inserted into a flask and 5 mL stannous chloride was injected through the syringe needle. The flask was shaken slightly. As the mercury was volatilized from solution the absorption signal, monitored on the strip chart recorder, increased, reached a maximum and returned to baseline. The sample flask was removed and replaced with a flask containing deionized distilled water. The system was flushed for a few seconds and then the next sample flask was attached. A calibration curve was prepared by plotting peak height versus micrograms of mercury.



REACTION VESSEL FOR COLD VAPOR DETERMINATION OF MERCURY

FIGURE 17: REACTION FLASK FOR THE DETERMINATION OF MERCURY BY CV-AAS. REDUCING AGENT IS ADDED TO THE FLASK VIA THE SYRINGE. Hg VAPOR IS LIBERATED FROM SOLUTION BY SPARGING WITH AIR. Hg-LADEN AIR IS PASSED INTO THE QUARTZ "T" ATOMIZER FOR MEASUREMENT.

f. Procedure for the Extraction of Methylmercuric Chloride from Water

The extraction of methylmercury chloride from water into benzene and the back-extraction of CH_3HgCl from benzene into aqueous cysteine solution were studied. Extractions were carried out in 60 mL separatory funnels. The following solutions were studied. Ten milliliters of aqueous CH_3HgCl (1 ppm Hg) were acidified with 15 ml conc HCl and extracted with 25 ml benzene. Ten milliliters of aqueous CH_3HgCl (1 ppm Hg) plus 15 ml cysteine solution (described in Chemicals and Reagents section) were extracted with 25 ml benzene. Twenty-five milliliters of CH_3HgCl (2 ppm Hg) in benzene were extracted with 10 ml deionized distilled water plus 15 ml cysteine solution. Blank extractions with benzene of 10 ml deionized distilled water plus 15 ml of either conc HCl or cysteine solution were carried out. A 30 min. extraction period was used and solutions were shaken frequently during this period. At the end of the extraction period, the phases were drawn off into separate glass bottles. Centrifugation was used to ensure complete separation of phases when necessary. Extractions were performed just prior to analysis. The mercury content of each phase was determined by either carbon disk or direct injection into the quartz "T" atomizer. All analyses were carried out at 253.7 nm.

g. Miscellaneous Tests for Mercury in Water

A number of other analytical methods were used in an

attempt to confirm the mercury concentrations found in water by the quartz "T" AAS method.

Classical dithizone colorimetry was attempted[21] but the detection limit, 0.5 ppm Hg^{2+}, was not low enough to permit direct detection of mercury in water. The Feigl[128] CuI spot test for Hg and HgX_2 (X = halide) was run on mercury standards and tap water. Aqueous solutions of Hg°, $HgCl_2$ and CH_3HgCl gave positive tests (development of a salmon color), but only at the 10 ppm Hg level. Therefore, the sensitivity was not adequate for water analysis.

Various methods were used in an attempt to remove mercury from tap water. Water was boiled in the hope of volatilizing the mercury present. Water samples were electrolyzed for 1 week at a potential of 1 V between spectroscopic grade carbon electrodes. Other samples were passed through columns of Amberlite IR-120 cation exchange resin, activated charcoal and dithizone-treated molecular sieve (Grace Chemicals, Type 13X) in an attempt to adsorb mercury from solution. Water was treated with thioacetamide, HCl and heat to evolve H_2S and precipitate HgS. Heat-cleaned activated carbon pieces (1 cm length, 0.63 cm O.D.) were placed in Erlenmayer flasks which contained 10 mL of water or Hg^{2+} standard. The flasks were allowed to stand for 48 hours to adsorb mercury from the water onto the carbon. A flask containing tap water without activated carbon was also allowed to stand for 48 hours. The carbon pieces were analyzed by dropping

them into the atomizer. The water was analyzed by direct injection.

The results of these tests will be presented and discussed in the following sections.

C. RESULTS

1. Methods of Calibration

The in-house and commercially prepared aqueous mercuric chloride standards gave identical calibration curves at 184.9 nm and 253.7 nm. Aqueous standards, introduced as 1 μL aliquots on carbon disks, were in excellent agreement with calibration curves prepared by injection, via a gas syringe, of air saturated with mercury vapor, after the aqueous standards were corrected for absorption signals from the deionized distilled water. This indicated that atomization was very efficient for $HgCl_2$ and that no matrix effect from water existed.

A mercuric chloride "unknown," prepared by independently by co-worker T. Ekman, was successfully analyzed by the carbon disk method using both aqueous and vapor calibration curves. The 0.5 ppm Hg "unknown" was found to contain 0.49 ppm Hg by this method.

Calibration curves were linear up to 30 ng Hg at 253.7 nm and up to 4 ng Hg at 184.9 nm. Correlation coefficients were invariably greater than 0.950. At 184.9 nm, calibration curves deviated considerably from linearity above 4 ng Hg.

2. Precision of the Method

The precision of the mercury vapor injections with the Hamilton gas syringe was determined by making 20 injections of

0.05 mL aliquots. This volume of air saturated with mercury vapor contained 0.71 ng Hg at the ambient temperature on the day of analysis. The mean absorbance (resonance minus background), at 184.9 nm, of the 20 aliquots was 0.3678 $\pm$ 0.0254 (mean $\pm$ σ). The relative standard deviation of the vapor injections was 6.9%.

The precision of the carbon disk technique was measured by making 20 injections of 1 µL aliquots of tap water delivered with the Hamilton microliter syringe onto Graphoil disks. The mean absorbance of 1 µL aliquots of tap water at 184.9 nm was 0.2088 $\pm$ 0.0372 (mean $\pm$ σ). The relative standard deviation of the carbon disk technique was 17.8%.

The precision of the direct injection technique using the Drummond microdispenser was determined by making 20 injections each of 1 and 2 µL aliquots of a 2 ppm Hg standard. The mean absorbance of 1 µL injections was 0.1733 $\pm$ 0.0260 (mean $\pm$ σ). The relative standard deviation was 15%. The mean absorbance of 2 µL aliquots was 0.3524 $\pm$ 0.0318 (mean $\pm$ σ). The relative standard deviation was 9.0%. This demonstrated clearly that direct injection of 1 µL aliquots was more imprecise than injection of 2 µL sample volumes.

3. Concentration Range of Mercury in Water

a. Laboratory Water Supply

Deionized distilled water and tap water samples from this laboratory were analyzed on 63 occasions during a period of eighteen months. Typical absorption traces are shown in Figures

18 and 19.

Deionized distilled water was found to contain 0.46 ± 0.38 ppm Hg (mean ± σ). Twenty-nine percent of the samples had mercury concentrations below the detection limit of the method, 0.05 ppm Hg. The range of concentrations was from <0.05 to 1.6 ppm Hg.

Tap water from several faucets within this laboratory was found to contain 0.40 ± 0.33 ppm Hg (mean ± σ). Seventeen percent of the samples contained no detectable amount of mercury. The distribution of mercury concentrations in tap water from this laboratory is shown in Figure 20. A concentration range of <0.05 - 1.20 ppm Hg was found. Concentrations, analytical wavelength and method of sample introduction are presented in Table 3.

b. Other Water Supplies

Fourteen private residences in Baton Rouge were sampled. The range of mercury concentrations in these samples was from <0.05 to 1.25 ppm Hg, with an average concentration of 0.65 ± 0.44 ppm Hg (mean ± σ). The distribution of concentrations is shown in Figure 20.

Tap water samples from Arkansas, lake water from Lake Maurepas, Louisiana, water from the Gulf of Mexico at Ft. Walton Beach, Florida and two varieties of commercial bottled spring water were found to contain no detectable amount of mercury. One sample of bottled drinking water contained 0.12 ppm Hg. Untreated and finished well waters from the Baton Rouge Water Works were found to contain 0.11 and 0.15 ppm Hg, respectively. Deionized

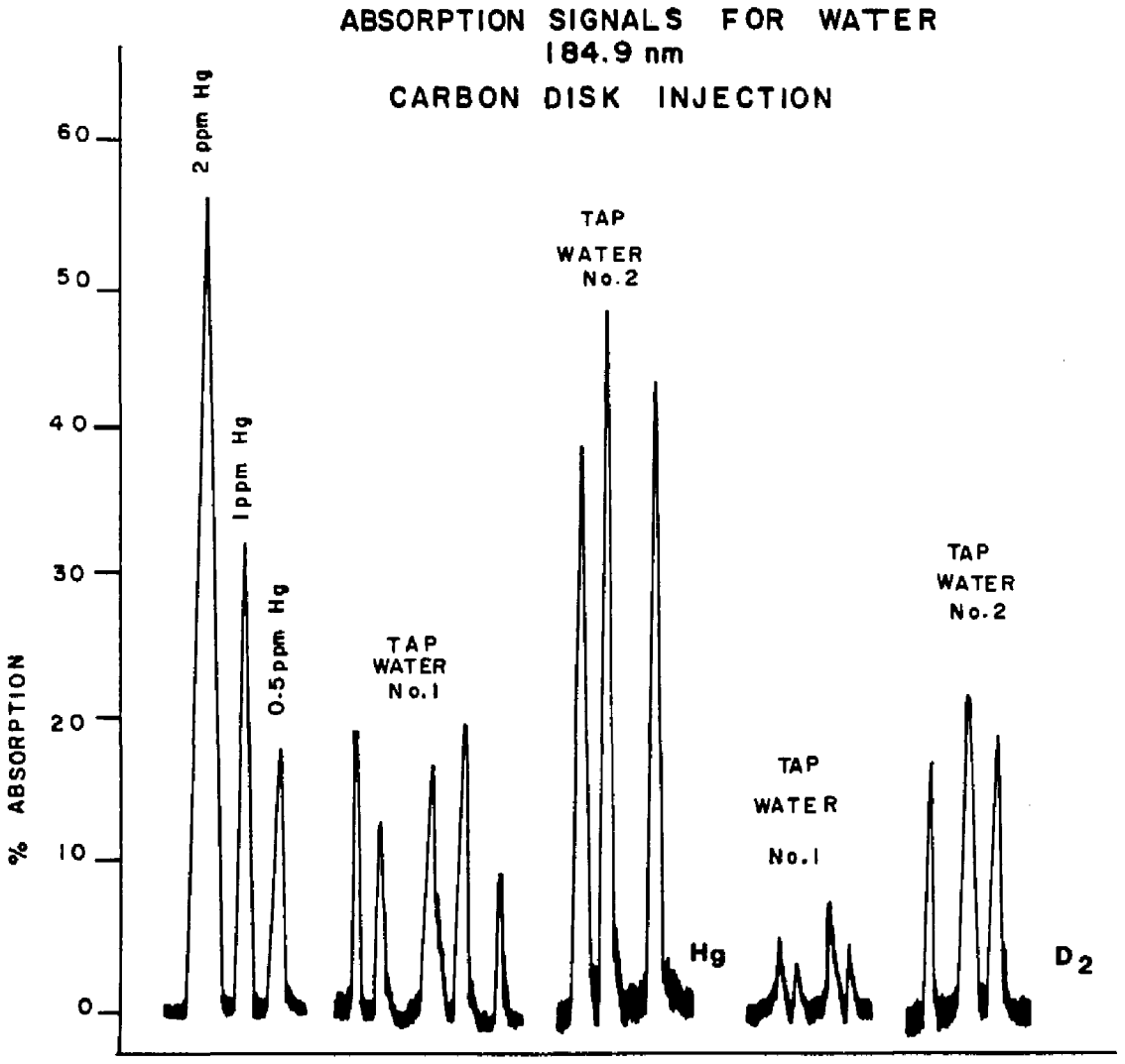


FIGURE 18: ABSORPTION SIGNALS FROM WATER AT 184.9 nm. TYPICAL ABSORPTION SIGNALS FROM 1 μL ALIQUOTS OF WATER INTRODUCED ON CARBON DISKS ARE SHOWN. RESONANCE (Hg) AND MOLECULAR BACKGROUND ABSORPTION (D_2) FOR TWO DIFFERENT SAMPLES ARE SHOWN, AS WELL AS SIGNALS FOR AQUEOUS MERCURY STANDARDS OF 0.5-2.0 ppm.

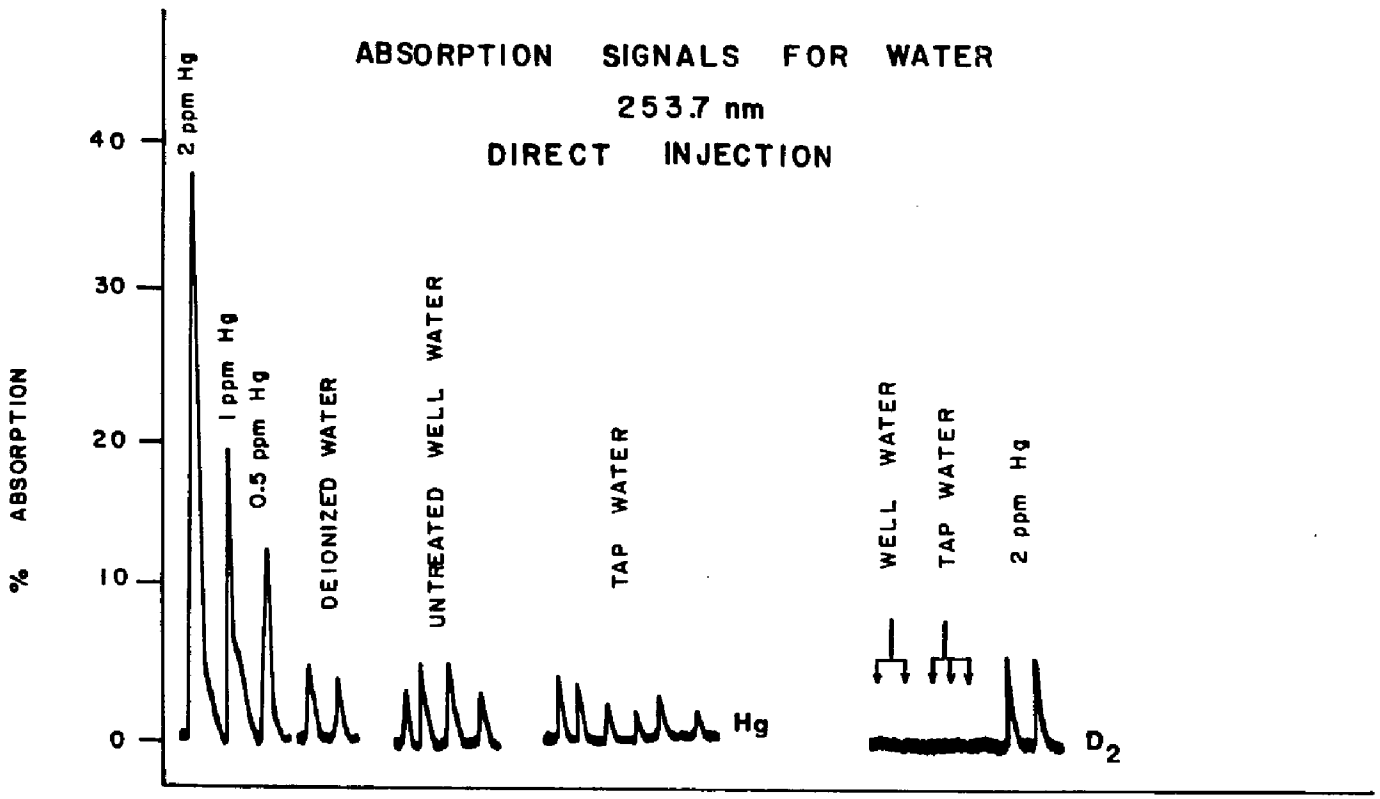


FIGURE 19: ABSORPTION TRACES OF WATER AT 253.7 nm. TYPICAL ABSORPTION SIGNALS FOR 2 μL ALIQUOTS INJECTED DIRECTLY WITH THE DRUMMOND MICROPIPETTE ARE SHOWN. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION SIGNALS FOR DEIONIZED, GROUND AND TAP WATER AND AQUEOUS MERCURY STANDARDS ARE SHOWN.

Table 3

Concentration of Mercury in Laboratory Tap Water

Date	(nm) Wavelength	Method of Introduction	ppm Hg	Average Hg Concentration (ppm) for Month
01/19/81	184.9	CD	0.23	JAN81 = 0.31
01/20/81	184.9	CD	0.40	FEB = ND[b]
03/31/81	253.7	CD	<0.05	MAR = <0.08
04/05/81	253.7	CD	0.21	APR = 0.23
04/28/81	184.9	CD	0.30	
04/30/81	184.9	CD	0.17	
05/13/81	184.9	CD	0.40	MAY = 0.70
05/26/81	184.9	CD	0.98	
05/29/81	184.9	CD	0.72	JUNE = ND[b]
07/01/81	253.7	CD	0.10	JULY = 0.41
07/07/81	253.7	CD	0.25	
07/08/81	253.7	CD	0.20	
07/09/81	184.9	CD	0.60	
07/13/81	253.7	CD	0.50	
07/14/81	184.9	CD	0.09	
07/15/81	184.9	CD	0.15	
07/20/81	184.9	CD	1.10	
07/27/81	184.9	CD	0.66	
08/12/81	253.7	CD	0.20	AUG = 0.45
08/15/81	253.7	CD	0.40	
08/20/81	253.7	CD	0.40	
08/30/81	253.7	CD	0.80	
09/30/81	253.7	CD	0.45	SEPT = 0.45
10/14/81	253.7	CD	0.40	OCT = 0.40
11/02/81	253.7	CD	0.55	NOV = 0.58
11/04/81	253.7	CD	0.80	
11/11/81	253.7	CD	0.70	
11/16/81	253.7	CD	1.20	
11/18/81	253.7	CD	0.90	
11/20/81	184.9	CD	0.40	
11/25/81	253.7	CD	<0.05	
11/28/81	253.7	CD	<0.05	
11/30/81	253.7	CD	0.70	
12/14/81	253.7	CD	0.75	DEC = 0.67
12/15/81	253.7	CD	0.60	
12/16/81	253.7	CD	0.66	
01/20/82	253.7	CD	1.25	JAN82 = 0.60
01/21/82	184.9	CD	1.05	
01/22/82	253.7	CD	0.88	
01/27/82	253.7	CD	0.65	
01/29/82	253.7	CD	0.40	
02/01/82	253.7	CD	0.40	FEB = 0.27

Table 3 (continued)

Date	(nm) Wavelength	Method of Introduction	ppm Hg	Average Hg Concentration (ppm) for Month
02/02/82	253.7	CD	0.35	
02/03/82	253.7	CD	0.30	
02/05/82	253.7	CD	0.70	
02/14/82	253.7	DM	0.07	
02/15/82	253.7	DM	0.10	
02/17/82	253.7	DM	0.51	
02/19/82	253.7	DM	<0.05	
02/20/82	253.7	DM	<0.05	
03/01/82	253.7	DM	<0.05	MAR = 0.10
03/03/82	253.7	CD	<0.05	
03/05/82	253.7	DM	<0.05	
03/10/82	253.7	DM	<0.05	
03/11/82	253.7	DM	0.57	
03/17/82	253.7	DM	0.20	
03/18/82	253.7	CD	0.21	
03/22/82	253.7	DM	<0.05	
03/24/82	253.7	DM	<0.05	
04/02/82	253.7	DM	0.13	APR = 0.13
04/13/82	253.7	DM	0.15	MAY = ND[b]
04/15/82	253.7	DM	0.11	
06/02/82	253.7	DM	0.24	JUNE = 0.24

(a) CD = carbon disk; DM = direct injection with Drummond microdispenser.

(b) ND = not determined

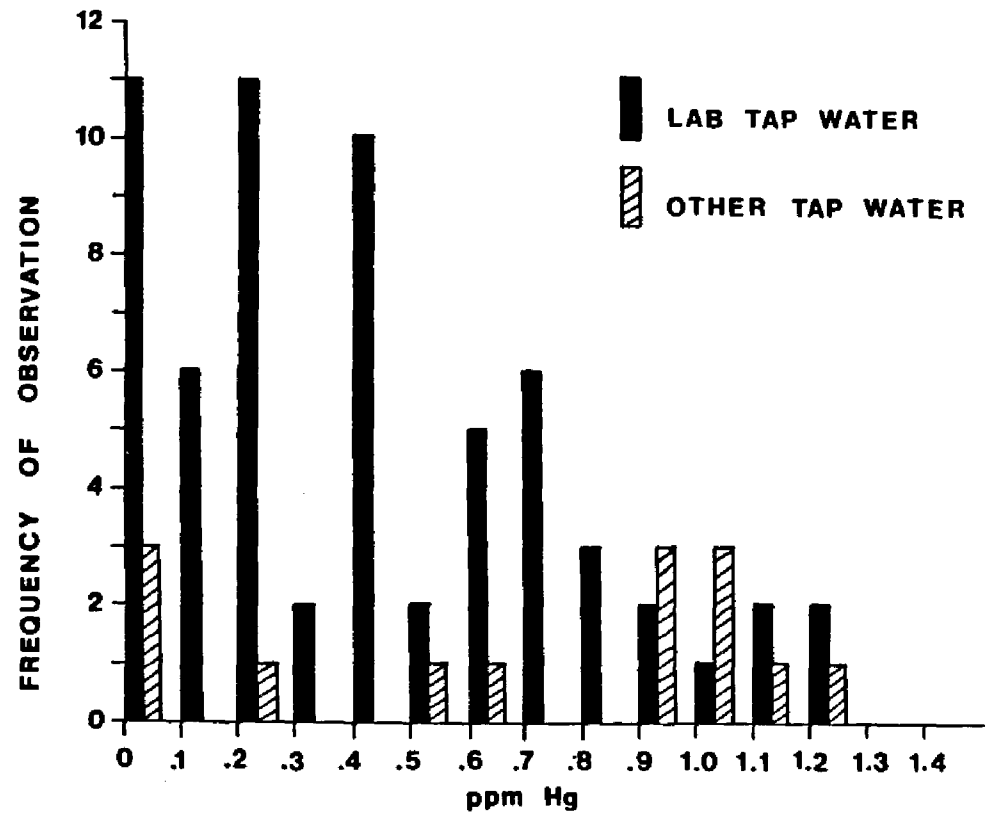


FIGURE 20: DISTRIBUTION OF MERCURY CONCENTRATIONS IN TAP WATER. SOLID BLACK BARS REPRESENT TAP WATER SAMPLES FROM THIS LABORATORY. STRIPED BARS REPRESENT OTHER TAP WATER SOURCES WITHIN THE CITY OF BATON ROUGE. CONCENTRATIONS WERE ROUNDED TO THE NEAREST 0.1 ppm Hg.

distilled water samples from two other research laboratories in the Chemistry Department were analyzed; mercury levels were below the detection limit of this method.

4. Possible Interference in the Direct Determination of Mercury in Water

An average concentration of 0.40 ppm Hg was found in laboratory tap water by direct analysis with the quartz "T" AAS system. This was higher than the 2 ppb limit for mercury in drinking water established by the US EPA. It was thought at first that this high concentration was due to some contaminant or interfering substance. A number of possible interferences were analyzed.

Absorption signals at 184.9 nm for 1000 ppm solutions of Fe^{3+}, Cu^{2+} and Zn^{2+} were exactly equal to the absorption signals of the deionized distilled water used to prepare these solutions. No interference was seen for 2000 and 5000 ppm Cl^- (as NaCl) solutions at 184.9 nm. A 1000 ppm solution of Si (as $NaSiO_3$) gave no interference at 184.9 nm.

The 10% HNO_3 used for cleaning glassware and equipment was found to contain about 0.4 ppm Hg, approximately the same as deionized, distilled water alone.

Hydrochloric acid, used in the CH_3HgCl extraction studies, presented two problems, mercury contamination and severe molecular absorption. A 6 M HCl solution, such as that used in the extraction procedure, gave a resonance absorption signal of about 50%

absorption at 253.7 nm. The 184.9 nm line could not be used, since 1 μL of 6 M HCl absorbed the entire line. The absorption signal at 253.7 nm was very broad and tailed badly. Approximately 30% of the peak height was due to molecular absorption. This background signal could be decreased to 5-10% absorption by neutralization of the HCl with $NaCO_3$. The same type of resonance and background signals exhibited by 6 M HCl were also seen on analysis of 5.25% aqueous sodium hypochlorite (Chlorox). Dilution of HCl to concentrations less than 2 M reduced the background signal to less than 5% absorption. The 6 M HCl solution was found to contain 3 ppm Hg.

5. Confirmatory Tests for Mercury in Water

Tap water which was boiled in an open beaker for 30 minutes was found to contain 0.83 ppm Hg. Fresh tap water analyzed at the same time contained 0.76 ppm Hg. No mercury appeared to be lost by volatilization from the boiled sample.

No difference in mercury concentration was found between fresh tap water and water electrolyzed at 1 V for one week. At the end of the electrolysis period, a waxy debris was found floating on top of the water. This was evidently due to decomposition of the polyethylene bottle which contained the water and electrodes.

Tap water containing 0.15 ppm Hg was run through 25 mL burets packed with either cation exchange resin or activated charcoal. Water eluted from either scrubber contained no detectable mercury

(<0.05 ppm). The mercury content of tap water was decreased by about 70% on passage through a 25 mL buret containing dithizone-treated molecular sieve.

No decrease in mercury concentration occurred on treatment of water with P_2S_5 or thioacetamide and heat in order to precipitate HgS.

Water samples and standards which had been treated with activated carbon for 48 hours were analyzed. The activated carbon pieces were also analyzed, by dropping them into the atomizer with tweezers. The absorption signals from the carbon pieces were very large (70-100% absorption) and broad. It was not possible to obtain quantitative information from these signals. Analyses of the water was more informative. Tap water treated with activated carbon contained 0.09 ppm Hg, fresh tap water contained 0.72 ppm Hg and tap water which stood for 48 hours without carbon contained no detectable mercury. Fresh deionized distilled water contained 0.36 ppm Hg while carbon-treated deionized water was found to have no detectable mercury. The 15 ppm Hg^{2+} standard contained only 4.3 ppm Hg after the treatment with activated carbon. The carbon appeared to adsorb about 87% of the mercury present in water (at a level of <1 ppm). About 71% of the mercury was adsorbed from the 15 ppm Hg standard. More than 93% of the original mercury content was lost from tap water on standing for 48 hours with no treatment whatsoever.

Tap water samples which had been allowed to stand over acid-washed mossy zinc for 4 hours were found to have no detectable amounts of mercury, but fresh tap water samples contained from 0.07 to 0.25 ppm Hg.

Finally, tap water was diluted with successive amounts of absolute ethanol, to make certain that the absorption signal was proportional to the amount of water. Dilutions of 5:0, 4:1, 3:2, 2:3, 1:4 and 0:5 (V/V) water/ethanol were analyzed. The mercury concentration decreased in a linear fashion as the amount of ethanol increased. The mercury concentration dropped from 0.48 ppm (pure water) to 0.16 ppm (pure ethanol) with successive dilutions.

6. Cold Vapor-AAS Analysis

Mercury standards of 0, 10, 50, 100, and 200 ng, which had been prepared in deionized distilled water, were analyzed by the cold vapor-AAS method. The mercury concentration in deionized distilled water was calculated by the method of standard additions from these standards. The absorption signals for the standards and samples were corrected for the reagent blank, which contained only the added reagents and no additional water. Mercury levels in tap water were determined from the calibration curve. A typical data set is given in Table 4. The detection limit for the CV-quartz "T" AAS system was estimated to be 0.01 ppb Hg at 253.7 nm.

Table 4

Cold Vapor-AAS Determination of Mercury in Water

Sample	Absorbance	ppb Hg
200 ng Hg	0.1722	2.00
100 ng Hg	0.1507	1.00
50 ng Hg	0.0580	0.50
10 ng Hg	0.0395	0.10
deionized water	0.0225	0.14[a]
reagent blank	0.0159	0.00[b]
tap water	0.077Y	0.83[c]

(a) Calculated by the method of standard additions from the line $Y = 0.0007X + 0.0106$, where Y = absorbance, X = ngHg. Correlation coefficient = 0.9963.

(b) Reagent blank absorbance was subtracted from all samples.

(c) Calculated from the above calibration curve.

Deionized distilled water was found to contain <0.01-0.14 ppb Hg by this method. Tap water was found to contain 0.29-0.83 ppb Hg. Tap water samples which were analyzed with and without the permanganate oxidation step appeared to contain the same amount of mercury. This would indicate that the mercury in tap water was in a chemical form which was readily reduced by Sn^{2+}.

7. Extraction of Methylmercuric Chloride

Solutions containing 1-2 ppm Hg as CH_3HgCl were extracted from aqueous HCl solutions into benzene and from benzene into aqueous cysteine solution to study the effectiveness of the extraction. Five replicate extractions were performed. The percent of added mercury recovered in both phases ranged from 87.5-119.0%, with an average recovery of 98.8%. At the 1-2 ppm Hg level, the extraction of CH_3HgCl from benzene into aqueous cysteine is 60-77% complete.

The distribution coefficient for extraction of CH_3HgCl from HCl into benzene was calculated to be 13.9; that for extraction of CH_3HgCl from benzene into aqueous cysteine, 0.36.

A summary of the data is given in Table 5.

D. DISCUSSION

1. Advantages of the Method

The use of the quartz "T" atomizer permitted the direct analysis of water samples for mercury. The elimination of pretreatment and preconcentration steps greatly increased the accuracy, simplicity and speed of the determination.

Table 5

Extraction of CH_3HgCl with Benzene

System	ppm Hg (as CH_3HgCl)[c] initial	final	% Hg recovered	K_D
1. cysteine/benzene				
a. aqueous phase	0	1.34	87.5	0.31
organic phase	2	0.41		
b. aqueous phase	1	1.12	119.0	0.42
organic phase	0	0.47		
2. HCl/benzene				
a. aqueous phase	0	0.19	94.5	8.95
organic phase	2	1.70		
b. aqueous phase	1	<0.05	94.2	>18.8
organic phase	0	0.94		

(c) Average of five replicate analyses of each system.

The high atomization efficiency of the quartz "T" ensured that all chemical forms of mercury in the sample were detected, which also increased the accuracy of this determination.

The absolute sensitivity was excellent, 10^{-10} g Hg at 253.7 nm and 10^{-11} g Hg at 184.9 nm. The absolute detection limit of this method exceeded that of most commonly used methods by a factor of 10-100, as can be seen in Table 6. However, the volume of sample injected was limited to 1-2 µL by several factors. The size of the absorption signal was a limiting factor at 184.9 nm. The size of the atomizer and flow rate through it restricted the amount of hot, rapidly-expanding sample gas which could be contained in the absorption cell. The size of the carbon disks limited the amount of sample which could be placed on them. As a consequence of this restriction of sample volume, the detection limit expressed in terms of concentration was not very impressive, only 50 ppb at 253.7 nm and 10 ppb at 184.9 nm.

The quartz "T" AAS system was able to exploit the very sensitive resonance line at 184.9 nm, in the vacuum-ultraviolet region of the spectrum. This was done by the simple expedient of purging the optical light path with non-absorbing nitrogen.

2. Calibration and Precision

The vapor calibration method was simple, rapid and did not contribute to a build-up of carbon disks in the atomizer. Vapor standards were also more reliable than aqueous standards since they did not deteriorate on storage. This method would have been

Table 6

Detection Limits of Instrumental Methods Applied to Determination of Mercury[129,130]

Method	Detection limit (ng Hg)
Flame AAS	20
Cold vapor AAS	1
Flame atomic fluorescence	3
Atomic emission	1-2
GC-ECD	0.1
Mass Spectrometry (SSMS)	1
Colorimetry	50-200
Polarography	50-1000
NAA	0.1-0.5
Quartz "T" AAS (this study)	0.005[a],0.05[b]

(a) at 184.9 nm

(b) at 253.7 nm

preferred over aqueous calibration curves, but for one problem. It was noted during the studies on methods of sample introduction that the concentrations of mercury found in deionized and tap water varied with the delivery device used. This variation was often by a factor of 5-10, calculated from vapor calibration curves. At first, this was thought to be only a normal daily fluctuation of mercury concentration. Part of the reason for this fluctuation became apparent when benzene solutions of CH_3HgCl, injected directly with the Drummond microdispenser, were compared to aqueous Hg^{2+} and Hg° vapor calibration curves. The CH_3HgCl standards gave higher absorption signals than the Hg° standards. This implied one of the following: that CH_3HgCl was atomized more efficiently than atomic mercury vapor; that atomic mercury vapor was being ionized, resulting in smaller absorption signals than expected or that too much CH_3HgCl was being injected. The first two alternatives did not seem very plausible, but the third choice was investigated. Ten aliquots each of water and benzene were transferred onto pieces of filter paper with the Hamilton microliter syringe, the Drummond microdispenser and the Finnpipette. The droplets formed circular spots on the filter paper. The diameters of the circles were measured and the area of the droplet was calculated. A considerable discrepancy existed between the volumes ejected by the different devices. The average area of an aqueous 1 μL droplet from the Hamilton microsyringe was 0.21 ± 0.01 cm^2, while that for the

Finnpipette was 0.7 $\pm$ 0.09 cm^2, almost four times as large. The Drummond microdispenser delivered aqueous 1 µL droplets with an area of 0.25 $\pm$ 0.03 cm^2, but 1 µL droplets of benzene with an area of 0.30 $\pm$ 0.03 cm^2, almost 20% greater than the aqueous droplets. Therefore, it was clear that calibration standards had to be prepared in the same solvent as the samples and that standards and samples had to be injected in the same manner with the same syringe or pipette. Mercury vapor calibration curves apparently could be used in place of aqueous standards introduced on carbon disks, since the curves were equivalent, but could not be used for calculation of concentrations of mercury in benzene or of samples injected with the micropipette.

Calibration curves were prepared in the manner most closely matched to the samples. Benzene solutions were compared to organic mercury standards in benzene, for example. The same delivery device, either Hamilton microsyringe or Drummond microdispenser, was used to transfer samples and standards. This avoided the discrepancy in volume between devices which was described above. The use of solution standards for calibration did entail the risks of deterioration by adsorption onto the flask and by volatilization of mercury, solvent or both. This risk was kept to a minimum by preparing new stock standards every six months and working standards on a daily basis. Pre-equilibrated flasks were used to prepare working standards to minimize adsorption onto the walls of the flasks.

Calibration curves at 184.9 nm were observed to deviate from linearity when more than 4 ng Hg was injected, as can be seen in Figure 12. This phenomenon was observed with mercury electrodeless discharge lamps[107] as well as with the demountable hollow cathode lamp used in this study. This deviation from linearity can be attributed to self-reversal in the lamps,[64] and broadening of emission lines due to hyperfine splitting and isotope effects.[131]

The relative standard deviation was 9-18% at the 1-2 ppm Hg level, and depended on the delivery device and aliquot volume. This precision was considered to be quite adequate for the purpose, especially when such small volumes were measured. The precision was also a function of the way in which the sample reacted with the bed. Differences in peak height and shape were noted which were dependent on how the carbon disk fell onto the bed. Samples which landed edge-on or face-down burned faster and gave sharper, narrower peaks than disks which landed sample side-up. Since only peak height, not area, was measured, this directly affected the precision. This was clear from the precision data. The carbon disk technique gave a relative standard deviation of 17.8%, while the direct injection technique, in which samples hit the bed in a more reproducible manner, gave a relative standard deviation of 9-15%.

Precision could have been increased if a double-beam optical system had been available. The current single-beam system

required measurement of resonance and background absorption signals on separate aliquots of sample. This was not as precise as the simultaneous background correction which could have been obtained through use of a double-beam system.

3. Choice of Resonance Line

The mercury resonance lines at 184.9 nm and 253.7 nm were used to measure absorption by the samples. The 184.9 nm line was more sensitive than the 253.7 nm line, but not by the factor of 50 expected from the difference in oscillator strengths of the lines. The sensitivity was only increased tenfold, due to a combination of factors. The noise level at 184.9 nm was much greater than that at 253.7 nm because the photomultiplier tube was operating at the lower limit of its response. In addition, the amplifier gain was set for maximum sensitivity in order to compensate for absorption of the line as it passed through the 20 cm of optical path exposed to air. Both of these factors contributed to electronic noise, and consequently, to a decreased detection limit, defined as a signal to noise ratio of 2.0.

The detection limit at 184.9 nm was limited by the molecular background absorption signal. This signal was due to H_2 and CO formed on combustion of the sample matrix. A 1 µL aliquot of water generated a 20% background absorption signal and a 40% resonance absorption signal. The size of the aliquot could not be increased because the absorption signal would exceed the linear working range. Although better sensitivity was obtained at

184.9 nm, the detection limit expressed as concentration was limited by the 1 μL sample volume, while at 253.7 nm, the detection limit could be increased by injection of up to 3 μL.

The 253.7 nm resonance line was useful in that no molecular absorption from the sample occurred at this wavelength, but peak heights for 2 μL aliquots were generally only 2-5% absorption. The error associated with measuring signals of 2-5% absorption is large.[132] Since neither line had a great advantage in the analysis of water, the wavelength used depended on the nature of other samples being analyzed that day.

4. Concentration of Mercury in Water

The concentrations of mercury found in local tap water were about two orders of magnitude higher than "allowable" concentrations. It is believed that the average concentration of 0.40 ppm Hg measured in water by this method did not represent a contaminated water supply, but, rather, an accurate estimate of the mercury which is normally present. As was demonstrated by cold vapor analysis of this same water supply, the current standard method is not accurate. Cold vapor analysis by the standard procedure measured less than 1 ppb Hg in tap water, well within the "allowable" limit which was based on the CV-AAS technique. Water analyzed by the quartz "T"-AAS direct method on the same days was found to contain 0.40-0.80 ppm Hg. As a result of direct analysis with the quartz "T" atomizer, it is believed that the cold vapor technique either did not measure all chemical

forms of mercury in the sample or permitted significant losses of mercury, possibly in the oxidation step.

It cannot be doubted that the substance measured in these studies was mercury. It is highly improbable that another substance would demonstrate atomic absorption at both of the mercury resonance lines. The substance was trapped by cation exchange resin, activated charcoal, activated carbon, dithizone and elemental zinc, as mercury would be. It did not appear to be precipitated by sulfide, which would indicate either that mercury was not present in water as Hg^{2+} or that the reagents themselves contained mercury. The experimental findings that the mercury in tap water was not lost by boiling or electrolysis supported the beliefs that the mercury was not simply ionic and was not very volatile (i.e. not Hg° or CH_3HgCl). It was probably in the form of a stable complex, or bound to bacteria, particulates or small organic molecules.

Mercury did not appear to be effectively removed by the mixed-bed deionizing column used in this laboratory. On many occasions, the mercury concentration in deionized water was significantly higher than that in tap water.

The natural water samples studied generally had mercury concentrations below the detection limit of this method. This was not surprising, since the most likely source of mercury in tap water is in the chloride added as a disinfectant. Most chlorine is contaminated with mercury as a result of its production by

mercury cell electrolysis of brine. This source of contamination will become less significant as more chlorine plants convert to processes which do not require mercury and as ozone treatment replaces chlorination in water purification.

An unexpected source of mercury in tap water was discovered in the laboratory. Water from one faucet consistently contained 2-3 ppm Hg, until a water aspirator brought over from the old chemistry building was removed from the faucet. The metal aspirator had evidently amalgamated mercury from water or air and had become grossly contaminated. The end of the aspirator was dipped into 10% nitric acid for a few minutes and the acid analyzed by the cold-vapor technique. A huge absorption signal, equal to >2000 ppm Hg, was generated. Water samples from this faucet were not included in the reported data.

Tap water samples from Arkansas contained no detectable mercury, but these samples were transported for 24 hr prior to analysis. It has been shown over and over again that dilute mercury solutions are not stable, so it is impossible to draw any conclusions from the lack of mercury in the Arkansas samples.

The amount of mercury consumed daily in drinking water can be estimated from the data presented. If it is assumed that 2 L of water containing 0.40 ppm Hg are consumed per day, then 0.8 mg Hg is ingested from this source.

The concentration of mercury in the laboratory's tap water appeared to vary on a daily basis. As can be seen from the data

in Table 3, the daily variations did not seem to follow any pattern. The average concentration of mercury in water for each month was calculated. These values are also listed in Table 3. The highest concentration, 0.70 ppm occurred in May, 1981. The concentration then dropped to about 0.4 ppm Hg through October, 1981, and rose to a peak of 0.68 ppm Hg in December, 1981. The concentration then dropped again in the first half of 1982. The significance of these observations cannot be evaluated, because, during several months, only one or two samples were analyzed and a few months were missed entirely.

5. Extraction of Methylmercuric Chloride

The standard extraction procedure for alkylmercury compounds appeared to be satisfactory, but it would certainly be advisable to run spiked samples during routine use of this method.

The percent recovery of methylmercury chloride from water varied from 87.5 to 119%. The recovery values greater than 100% may be due to some evaporation of the benzene layer with a resulting increase in mercury concentration.

The extraction efficiency of the procedure could be improved by multiple extractions, but these are not commonly used.

The determination of mercury in these extracted samples was complicated by the high background absorption from the hydrochloric acid used. Measurement of a small change in mercury concentration on top of such a large, broad background signal was very difficult. The molecular absorption could be greatly reduced

by neutralization of the acid with sodium carbonate, but the heat evolved during neutralization could cause loss of volatile CH_3HgCl.

E. CONCLUSIONS AND SUMMARY

1. It was shown that the use of the quartz "T" atomizer and sample introduction on a carbon disk or by direct injection provided a feasible and successful technique for the direct determination of mercury in water by atomic absorption spectroscopy.

2. The direct determination employed in these studies eliminated sample pretreatment and preconcentration steps. Positive and negative errors associated with these steps were therefore avoided.

3. The direct determination, coupled with the high atomization efficiency and flow-through design of the quartz "T", increased the accuracy of the analysis by ensuring atomization and detection of all chemical forms of mercury present in the sample.

4. The use of the quartz "T" atomizer permitted analysis at the more sensitive 184.9 nm resonance line, in the vacuum ultraviolet region of the spectrum, as well as at the spin-forbidden 253.7 nm resonance line.

5. Tap water from this laboratory contained 0.40 ± 0.33 ppm Hg. Deionized distilled water from this laboratory contained 0.46 ± 0.38 ppm Hg. It was evident that the mixed-bed ion exchange column used for deionization of water had no effect on the mercury concentration.

6. Other water sources in Baton Rouge were found to have an average mercury content of 0.65 $\pm$ 0.44 ppm Hg.

7. The standard method for the determination of mercury in water, cold vapor-atomic absorption spectroscopy, was shown to give results which were two orders of magnitude lower than those obtained by direct analysis. The cold vapor results are belived to be in error, most probably due to failure of the method to determine all chemical forms of mercury in the sample.

8. The extraction of methylmercury chloride from acidified aqueous solution into benzene was shown to be 90-100% complete. The distribution coefficient was calculated to be 13.9.

CHAPTER 2

THE DIRECT DETERMINATION OF MERCURY IN URINE

A. INTRODUCTION

Mercury is accumulated in the kidneys on prolonged exposure, especially exposure to mercuric compounds, and is eliminated slowly.[10] It has been known[133] since the 19th century that mercury is excreted in urine. Most of the early data were obtained from studies of patients exposed to Hg as a treatment for syphilis.

Urine has been used extensively to monitor industrial exposure to mercury because the samples are easy to collect and convenient for routine analysis. However, there is some disagreement as to the value of the data in assessing exposure.[10]

1. Urine as a Biological Indicator of Mercury Exposure

The excretion of mercury in urine has been found to depend upon the chemical form of the exposure. Inorganic Hg compounds were eliminated mainly in the feces and urine. High concentrations were found in both media from patients injected with $HgCl_2$ for treatment of syphilis; for example, Lomholt[134] found 25-30% of a 5 mg dose in 24 hour urine samples.

Excretion of ^{197}Hg after intravenous injection was studied by Sodee,[135] who found that 75% of the amount given was excreted in urine within 72 hours.

After inhalation of vapor containing 0.05-0.10 mg Hg/m^3,

workers were found[136] to excrete 0.12 mg Hg/day in urine. The amount of mercury in urine increased as its concentration in inhaled air increased.[136]

Alkylmercury compounds, on the other hand, were not excreted in urine to any great extent. After oral administration, only 10% of a dose of CH_3Hg^+ was eliminated in urine.[137] The main route of excretion for methylmercury compounds was via feces.[137]

The mechanism of excretion of mercury by the kidney into urine is not known: both tubular mechanisms and glomerular filtration are involved, but the available data are contradictory and do not allow a definite conclusion to be drawn.[10] Metals bound to low molecular weight proteins such as metallothionein (mol. wt. = 6500) can pass through the glomerular membrane and thus be cleared from blood plasma into the tubular fluid.[138] Mercury is known to bind to metallothionein[139] and in the kidney, most mercury is found in the tubular region.[140] This suggests that metallothionein-bound mercury is reabsorbed in the renal tubules, as is metallothionein-bound cadmium.[138]

What was clear from reported data was that urine mercury is an unreliable index of individual exposure to mercury, especially of exposure to alkylmercury.[10] Concentrations of mercury in urine fluctuated independently of exposure.[141] Wide diurnal and day-to-day fluctuations have been reported in individuals. Adjustment of results for the specific gravity of the specimen did not alleviate the problem.[10] No good correlation existed

between mercury levels in urine and either mercury exposure or mercury poisoning symptoms on an individual basis.[10] There was however, a positive association between mercury exposure and mercury concentrations in urine on a group (i.e., industrial, occupational) basis.[10,142]

Despite the fact that analysis of urine has often been used to evaluate exposure, there was very little data in the literature on "normal" mercury levels in a non-occupationally exposed population. A World Health Organization study of mercury in 1107 urine samples collected from around the world found[22] that 79% of the samples contained <0.5 μg Hg/L (analytical zero), and 95% contained <20 μg Hg/L. No influence was found by age, sex or residence (urban or rural) on mercury levels. The World Health Organization regarded 20 μg Hg/L as an upper normal limit for mercury in urine. This limit is almost certainly too low because the method used for analysis[22] (dithizone extraction followed by cold-vapor AAS) does not completely extract Hg from urine.[143] "Normal" levels of 7-20 μg Hg/L were reported by Clarkson and Greenwood,[144] who used an isotope exchange method which did not detect organomercury compounds. They reported that their data agreed with a published range of 10-50 μg Hg/L in normal urine.

2. Analytical Problems in the Determination of Mercury in Urine

Urine is a very complex sample. It contains a wide variety of inorganic ions in various concentrations, Na^+, K^+

and Cl^- being the most abundant.[145,146] Urine also contained significant amounts of organic compounds such as urea, uric acid and creatinine. The concentrations of these components change with the individual and with fluid intake. Some of the constituents of normal adult urine are listed in Table 7.

Mercury concentrations in urine are very low (ppm or less) and the chemical forms of mercury in urine are not known. The high salt concentrations and variable concentrations of organics can cause serious matrix effects in analysis. In particular, chlorine compounds and many of the organics exhibit molecular absorption at the mercury resonance lines, 184.9 and 273.7 nm, in atomic absorption analysis. Also, Co is a direct spectral interferent at 253.7 nm. Most analytical methods require preliminary wet acid digestion of urine, which can result in loss of mercury through volatilization or contamination from added reagents.

Bacterial action in stored urine samples has been shown[147] to enhance the volatility of mercury in urine. More mercury was lost on addition of concentrated H_2SO_4 to aged urine specimens than to fresh specimens. This phenomenon was not observed in urine specimens which were treated with bactericides and then aged. It is well-known that bacteria can reduce Hg^{2+} and Hg^{+} to Hg° and can methylate Hg^{2+}, Hg^{1+} and Hg° to form methylmercury cation and dimethylmercury.[2,148] Hg° and alkyl mercury compounds are more volatile than inorganic mercury compounds. In addition, changes in the chemical form of mercury

Table 7

Selected Constituents of Normal Adult Urine

Constituent	Amount Excreted/Day
Solids	60.2 g
Water	1400 g
Calcium	231 mg
Chloride	7 g
Cobalt	0.005 mg
Copper	1.54 mg
Magnesium	94.5 mg
Mercury	0.7 µg
Phosphorus, inorganic	840 mg
organic	9.1 mg
Potassium	2.38 g
Selenium	35 µg
Sodium	4.2 g
Sulfur, total	1.12 g
Zinc	1.26 mg
Ammonia	0.7 g N
Bicarbonate	140 mg
Protein, total	2-70 mg
Creatinine	.3-.8 g N
Cystine, total	119 mg
Methionine, total	9.8 mg
S-methyl cysteine	1.47 mg
Urea	6-18 g N
Vitamin B	0.03 µg

Normal pH = 5.5 - 5.6
Normal 24 hour volume = 600 - 2500 mL

in urine may cause it to go undetected by some analytical procedures. For example, the electron capture detector commonly used for GC analysis of organomercury halides does not respond to dimethylmercury.

3. Common Methods for the Determination of Mercury in Urine

A wide variety of analytical methods has been employed in the determination of mercury in urine. Of historical interest are are the Reinsch test[149] and the micrometric method.[150] Both involved plating mercury out of urine onto a copper wire. In the first case, the mercury was determined gravimetrically; in the second case, the mercury was distilled off the wire into a capillary and the diameter of the mercury drop measured.

Up until the late 1960's, the recommended method for determination was dithizone extraction and spectrophotometric measurement of the mercury-dithizone complex.[147,151-153] A typical dithizone determination required digestion of the urine with H_2SO_4/HNO_3 or $H_2SO_4/KMnO_4$ under reflux with a trap for the distillate. Mercury was extracted by shaking with dithizone in CCl_4 or $CHCl_3$. Multiple extractions were often performed. To remove other metals which may have been extracted, the mercury-dithizonate was destroyed and the mercury back-extracted into aqueous thiosulfate. Interfering compounds such as Cu-dithizonate did not partition into the thiosulfate solution. The aqueous phase was then reoxidized and re-extracted with dithizone. The color of the mercury-dithizone complex was

measured immediately because the color changed rapidly. The dithizone method was cumbersome, tedious, lengthy and not very sensitive (detection limit = 0.5 μg Hg). The extraction of mercury by dithizone has been shown to be incomplete under certain digestion conditions.[143] Copper, which is present in urine at much higher levels than mercury, interferes in the determination and has to be eliminated.

Polarography[154] and X-ray fluorescence[155] have been used to determine mercury in urine. Atomic emission spectroscopy has been employed, with a modified Reinsch technique to collect the mercury in a suitable form.[156] The procedure called for digestion of the urine with HNO_3/H_2SO_4 under reflux and filtration of the digested solution through a column packed with copper dust. The mercury amalgamated with the copper dust, but the filtration had to be performed slowly to allow quantitative extraction. Each filtration required about 1 hour. The copper dust was packed into a carbon electrode and arced in an emission spectrograph. There were several problems with the use of atomic emission for mercury determinations. The amount of sample vaporized into the arc discharge varied from one sample to the next because of differences in physical packing and the size of particles packed into the electrode.[132] This problem usually was overcome by using an internal standard element with the same vaporization characteristics as the element to be determined.[132] Mercury was so volatile that there was no suitable element to

serve as an internal standard. Another problem arose from the volatility of mercury: no preburn time can be used. When a sample was introduced into an electrical discharge, the intensity of emission was erratic and difficult to control.[132] If accurate results were desired, the emission was not recorded for a short period of time (the preburn time) until the signal became steady.[132] Mercury was too volatile to incorporate a preburn time into the analysis. Both the lack of preburn time and the lack of an internal standard severely limited the accuracy of mercury determination by atomic emission.

Clarkson and Greenwood[144] used an isotope exchange method to determine mercury in urine. Tracer quantities of ^{203}Hg were added to undigested samples and were equilibrated with stable ^{200}Hg vapor passed through the sample. Rapid isotope exchange was reported to occur. The equilibrated mercury vapor was collected on a tube of activated Hopcalite located in the well of a gamma counter. The increasing activity was recorded and the half-time of exchange was directly proportional to the mercury concentration in the sample. The determination was suitable for Hg levels >10 ppb, but organic mercury compounds were not determined by this method.

The most common method in current use for urine mercury determinations was atomic absorption spectroscopy. A few flame-AAS procedures have been reported but most determinations used some variation of the cold-vapor technique.

A flame-AAS technique was reported by Berman[56] and is distributed by Perkin-Elmer in their AAS Methods Manual. Urine was treated with trichloroacetic acid (TCA) to precipitate the protein. The mercury remaining in the supernatant was extracted with ammonium pyrrolidine dithiocarbamate (APDC) into methyl-isobutyl ketone (MIBK) after adjustment of the pH to 2.8-3.5. The MIBK layer was aspirated into an oxidizing flame and atomic absorption of the mercury resonance line at 253.7 nm was measured. A sensitivity of 0.01 ppm was reported. A variation of this procedure was reported[157] in which the APDC-MIBK extract was placed in a tantalum boat. The tantalum boat was slowly pushed into the flame: the advantage of this technique was that most of the solvent evaporated before the sample entered the flame, reducing molecular background absorption. There were two major problems with the use of a TCA precipitation step. First, any protein-bound mercury in the urine sample was precipitated and not measured. Mercury was known to have a great affinity for sulfhydryl and nitrogen-containing groups, so there was a very good chance that some mercury in urine was protein-bound. In addition, work by Schulert et al.[158] showed that about 75% of added inorganic radiotracer mercury never left the initial TCA precipitate and that only 2-10% of the added inorganic mercury was recovered.

Numerous variations of the cold vapor-AAS method have been used to determine mercury in urine. Lidums and Ulfvarson[159]

used oxygen combustion to destroy the urine matrix. The resulting mercury vapor was trapped on gold and then released into a quartz absorption cell by heat. Absorption was measured at 253.7 nm. A sensitivity of 1 ppb was reported. Rathje[160] added 5 mL conc. HNO_3 to 2 mL urine and allowed it to stand for 5 minutes at room temperature. Stannous chloride was added to reduce the mercury and the solution aerated to sweep the mercury vapor into a quartz absorption cell. A detection limit of 3 ppb was claimed.

Gage and Warren[161] used different reducing conditions to measure selectively inorganic mercury and total mercury. Total mercury was released by the addition of stannous chloride to an acidified urine sample to which cysteine had been added. Only inorganic mercury was released under alkaline reducing conditions.

Kudsk[143] used wet oxidation of urine and extraction of mercury as the dithizonate before determination by CV-AAS. He noted problems with incomplete extraction of mercury under oxidation conditions which did not destroy all of the organic material in the samples. A detection limit of 0.02 μg was reported.

Speciation of organomercurials in urine has been performed by GC with an EC detector after extraction of mercury with benzene. Cappon and Smith[121] detected both inorganic and organic mercury by GC-ECD. The organomercurials were extracted from urine with benzene. The inorganic mercury left behind in the aqueous phase was methylated with tetramethyl tin and then extracted into benzene. The two benzene extracts were back-extracted with

thiosulfate and the organomercury compounds isolated as the bromide derivatives, RHgBr (R = alkyl, aryl substituent). Both extracts were chromatographed. A detection limit of 1 ppb was claimed. The accuracy of the procedure was checked by measurement of radiotracer spiked samples by liquid scintillation spectrometry. Total mercury recovery varied from 75-90%.

4. Need for an Improved Analytical Procedure

It can be seen that all the reported methods for the determination of mercury in urine have significant disadvantages. The method developed for the direct analysis of mercury in water could be applied easily and effectively to the direct determination of mercury in urine. This technique eliminated both positive errors due to added reagents and negative errors inherent in extraction procedures.

This chapter describes the application of the carbon disk technique and direct injection onto the carbon bed to urine samples.

B. EXPERIMENTAL

1. Equipment

The equipment used in the analysis of urine samples was identical to that described in Chapter 1. Both the 253.7 nm and 184.9 nm resonance lines were used; operating conditions were the same as those previously employed.

2. Procedure

a. Sampling Techniques

Spot urine samples were collected at random from

individuals who were not occupationally-exposed to mercury. All subjects sampled were members of the university population. Two subjects were studied over a period of eight months: each of their urine specimens was collected following a period of exercise and a sauna. Samples were collected in polyethylene vials which were cleaned previously in HNO_3 and rinsed well with distilled deionized water. All samples were analyzed within three hours of collection. The average volume of urine collected was 5 mL.

b. Determination of Hg in Urine

Urine samples were introduced into the atomizer in two ways, on a carbon disk or by direct injection. The carbon disk technique consisted of placing one microliter of urine on a cleaned carbon disk using a Hamilton microliter syringe. The disk was dropped onto the heated carbon bed where decomposition and atomization occurred. Direct injection of 1 or 2 microliters of sample onto the heated carbon bed was also used. The modified Drummond micropipette was used for sample injection.

Absorption measurements were made at the mercury resonance lines at 253.7 nm or 184.9 nm while background absorption was measured with a deuterium lamp at the same wavelengths. The blank absorbance by carbon disks was measured and subtracted when necessary from the sample absorbance.

c. Calibration Procedure

Calibration curves were established by measuring the absorption of aqueous $HgCl_2$ standards. The standards were

introduced into the atomizer in the same manner as the samples, either on a carbon disk or injected directly. The standard addition method was used to check for matrix effects from the urine. To prepare the standard additions, 1-10 microliter amounts of a 100 ppm aqueous mercury (as $HgCl_2$) standard were added to 0.5 mL aliquots of urine. The slopes of the aqueous calibration curve and the standard addition calibration curves were equal, as can be seen in Figure 21. Also, standard addition curves prepared from urine of different subjects were equal in slope. Therefore, no matrix effect was evident and subsequent sample absorbance was compared to aqueous calibration curves. Calibration standards were introduced into the atomizer in the same manner as samples were introduced, by direct injection or by carbon disk injection. Typical absorption signals from standards and samples are presented in Figures 22-24. As can be seen, a higher, narrower absorption peak was generated by direct injection than by carbon disk injection. This is believed to be due to the slower heating of the sample on the carbon disk and to the tendency of the sample to soak into the carbon disk. Time was required for the sample to diffuse back out of the disk. Therefore it was necessary to perform standard and sample analysis using the same injection technique.

It can also be seen that no molecular background absorption occurred at 253.7 nm (Figure 23 and 24) while molecular background signals of 10-20% absorption were recorded at 184.9 nm (Figure

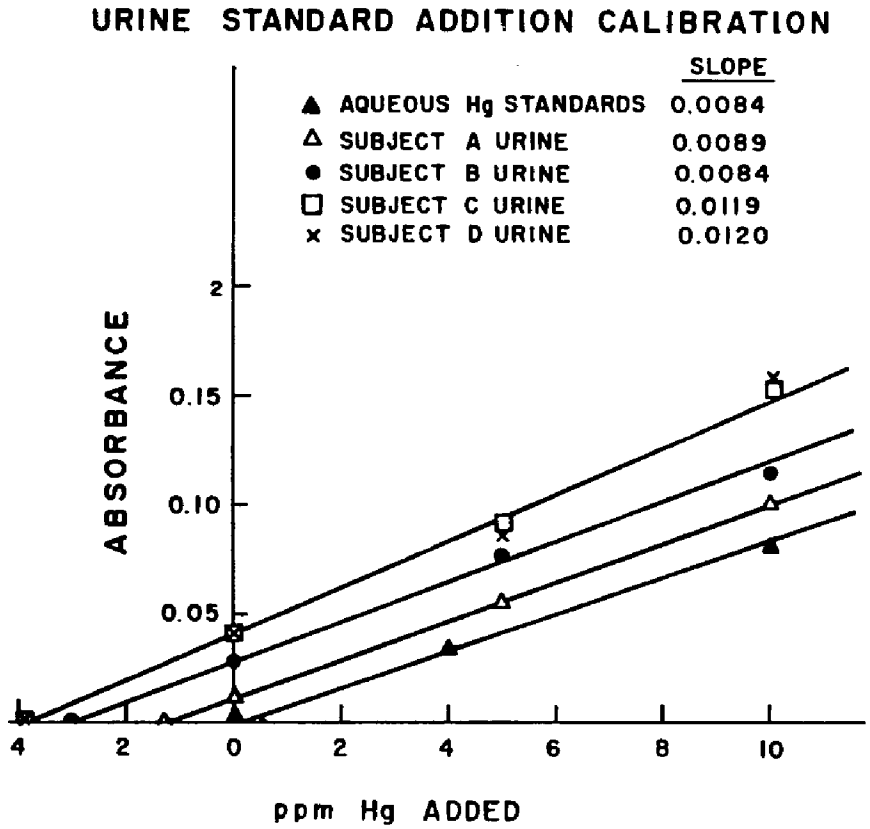


FIGURE 21: TYPICAL CURVES OBTAINED FROM ANALYSIS OF URINE BY THE METHOD OF STANDARD ADDITIONS. SAMPLES WERE INTRODUCED INTO THE ATOMIZER ON CARBON DISKS. THE SLOPES OBTAINED ON ANALYSIS OF URINE FROM FOUR INDIVIDUALS ARE COMPARED TO THE SLOPE OF THE CALIBRATION CURVE PREPARED FROM AQUEOUS MERCURIC CHLORIDE STANDARDS. SLOPES WERE SIMILAR ENOUGH TO ALLOW DIRECT COMPARISON OF URINE SAMPLES WITH THE AQUEOUS MERCURY STANDARDS.

FIGURE 22: TYPICAL ABSORPTION SIGNALS for URINE and $HgCl_2$ STANDARDS

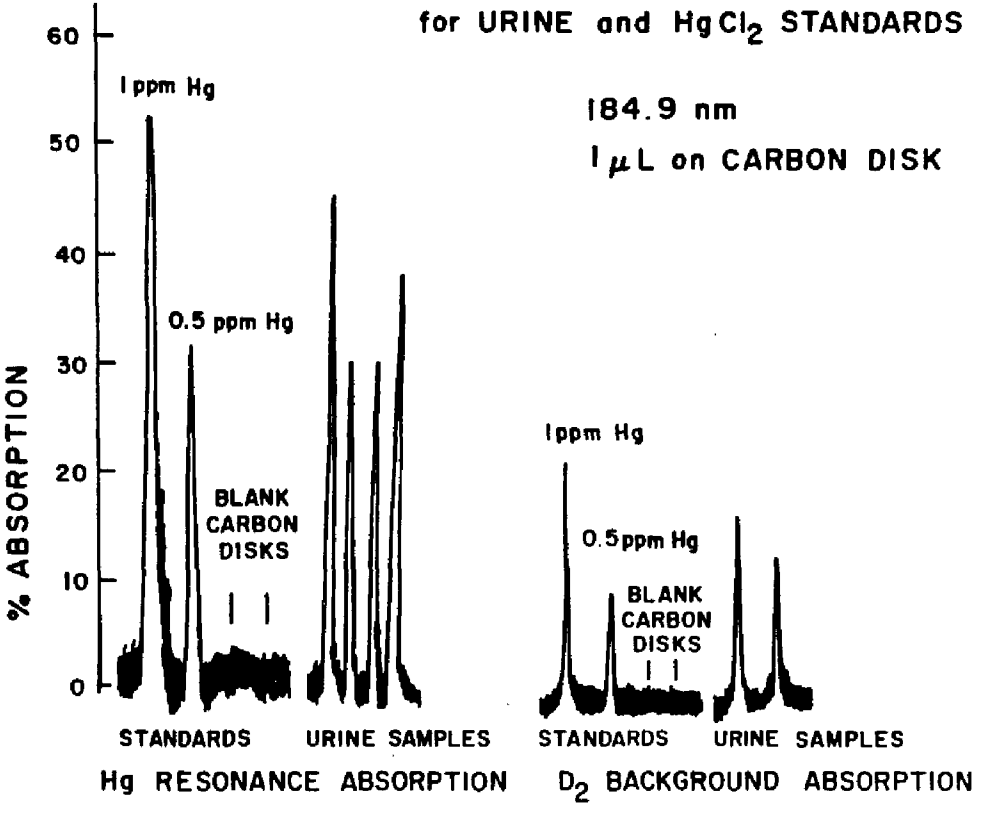


FIGURE 23: TYPICAL ABSORPTION SIGNALS FOR URINE & $HgCl_2$ STANDARDS

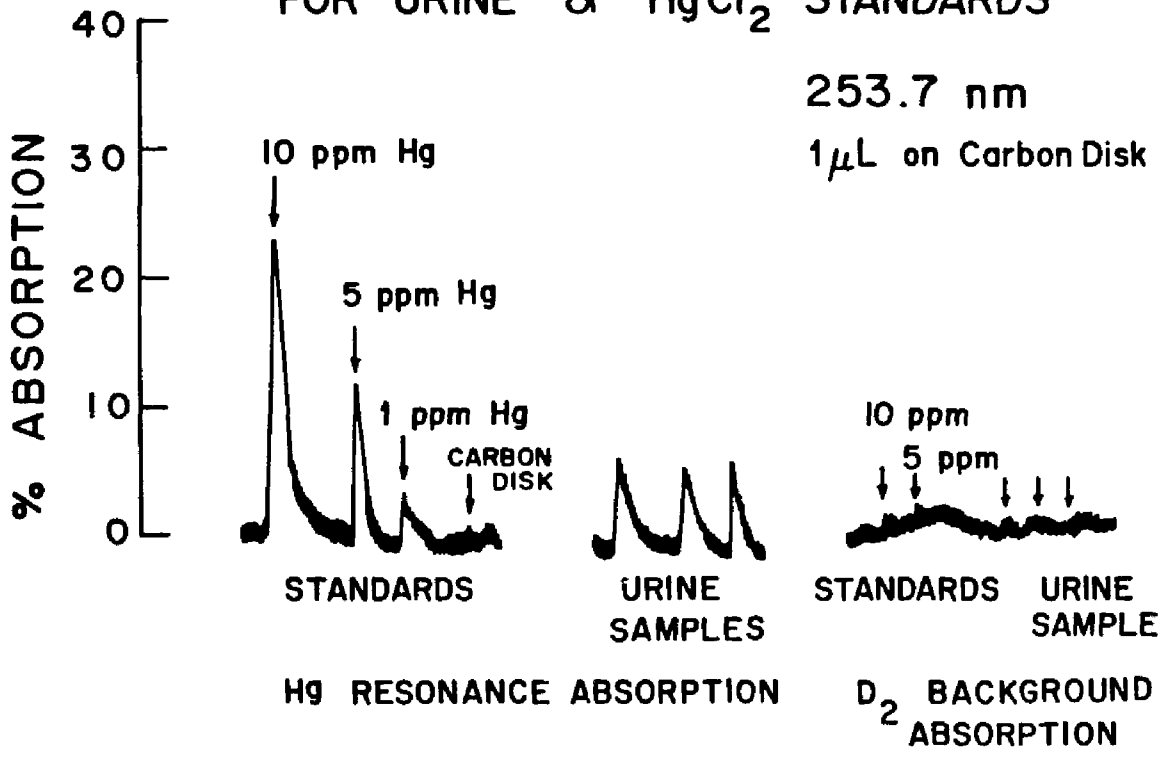
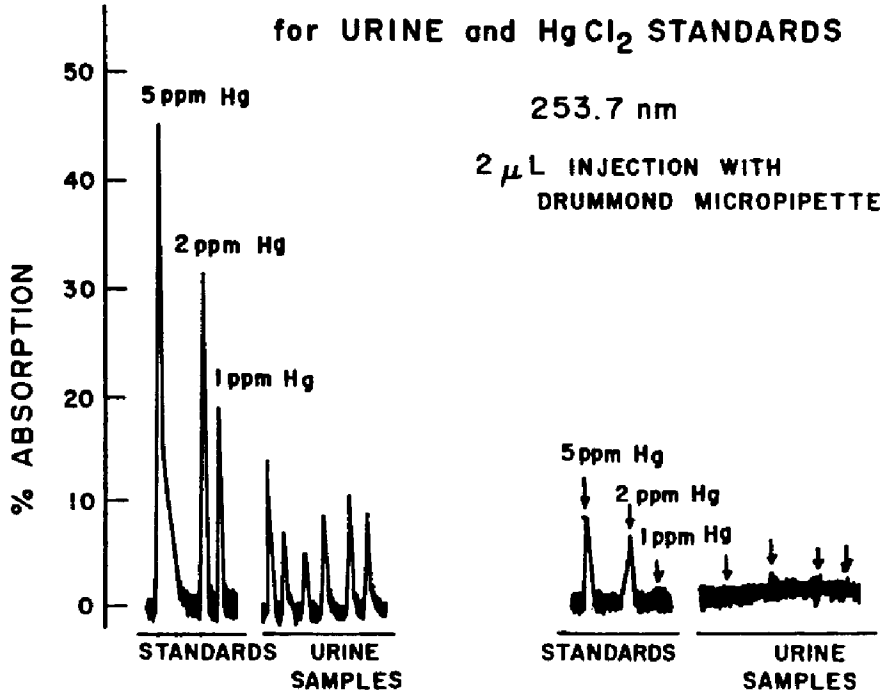


FIGURE 24: TYPICAL ABSORPTION SIGNALS for URINE and $HgCl_2$ STANDARDS



22). For this reason, all analyses were carried out at 253.7 nm.

The precision of the method was calculated by multiple analyses of a 2 ppm aqueous Hg standard. The relative standard deviation at this concentration was calculated to be 16%. The precision of the urine analysis is discussed later in this chapter.

The sensitivity of the method, defined as the amount of Hg equal to 1% absorption, was 1.0×10^{-10} g Hg at 253.7 nm.

C. RESULTS

1. Concentration Range for Mercury in Urine of a "Normal" Population

Random urine samples were analyzed from a number of non-occupationally exposed individuals. The results of the analysis are presented in Table 8. For 23 subjects, the mean concentration of mercury in urine was 1.3 ppm. For females, the mean value was 1.5 ppm Hg and for males, 1.2 ppm Hg. The mercury concentrations ranged from 0 (none detected) to 3.7 ppm. Figure 25 illustrates the distribution of the mercury concentrations found. To construct this curve, mercury concentrations were rounded to the nearest 0.5 ppm. There was no significant difference between the mercury concentrations in males and females.

2. Precision of the Method

Repetitive analysis of a single urine sample was performed to obtain a measure of the precision of the technique. The data

Table 8

Concentration of Hg in Urine of Normal Adults

Female ppm Hg	Male ppm Hg
3.7	2.3
1.3	2.6
1.2	0.6
2.9	N.D.
0.8	0.9
1.6	0.8
1.2	1.0
0.6	1.4
1.0	1.0
1.0	0.1
1.0	2.0
1.5	2.0
$n = 12$	$n = 11$
$x = 1.5$	$x = 1.2$
$\sigma = 0.9$	$\sigma = 0.8$
$\sigma^2 = 0.7$	$\sigma^2 = 0.7$

Overall

$n = 23$
$x = 1.3$
$\sigma = 0.9$
$\sigma^2 = 0.7$

N.D. = none detected (<0.05 ppm)

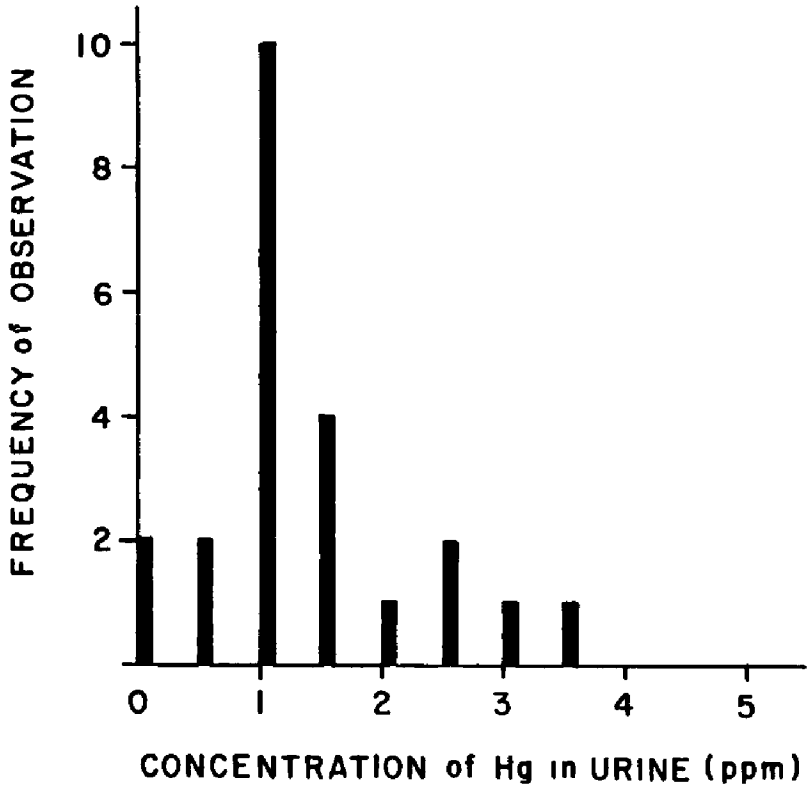


FIGURE 25: DISTRIBUTION OF MERCURY CONCENTRATIONS IN RANDOM URINE SAMPLES FOR A NON-OCCUPATIONALLY EXPOSED POPULATION. CONCENTRATIONS WERE ROUNDED TO THE NEAREST 0.5 ppm Hg.

collected are presented in Table 9. One urine sample was analyzed by direct injection and one by carbon disk injection. The relative standard deviations were 30% and 25% respectively.

3. Variations in Concentration of Mercury in Urine of an Individual

The urine of two male subjects was analyzed regularly for a period of eight months. Wide day-to-day fluctuations in mercury concentration were found, in agreement with data reported in the literature. The data accumulated are presented in Table 10 and the distribution of these values is illustrated in Figure 26.

The mean concentration for Subject 1 was 0.9 ± 0.8 ppm Hg and for Subject 2 was 0.8 ± 0.6 ppm Hg. It must be emphasized that the "standard deviations" calculated for these data are not an indication of precision of the method, but rather an indication of the amount of variation in urine mercury concentrations for each individual on a day-to-day basis. Each sample analyzed and reported in Table 10 was unique. The variations in mercury concentration among these samples were not due to random error, but to real differences among the specimens. The "standard deviation" serves only as a numerical indicator of the distribution of the urine mercury values within the observed range.

4. Changes in Concentrations of Mercury in Urine on Storage

A number of urine samples were kept for periods of up to five days from the time of collection to see the effect of storage

Table 9

Short Term Precision of Urine Analysis

2 µL Direct Injection, 253.7 nm

Urine A Aliquot #	% Absorption	Absorbance	ppm Hg	
1	8.5	0.0386	1.9	
2	8.5	0.0386	1.9	
3	4.0	0.0177	0.8	
4	6.8	0.0306	1.5	n = 10
5	6.0	0.0269	1.3	x = 1.3 ppm
6	5.2	0.0232	1.1	σ = 0.4
7	4.0	0.0177	0.8	σ^2 = 0.13
8	5.5	0.0246	1.2	R.S.D. = $\pm$ 30%
9	5.5	0.0246	1.2	
10	5.0	0.0223	1.1	

1 µL on Carbon Disk, 253.7 nm

Urine B Aliquot #	% Absorption	Absorbance	ppm Hg	
1	6.0	0.0269	2.1	
2	7.0	0.0315	2.4	
3	5.0	0.0223	1.7	
4	3.2	0.0141	1.1	n = 10
5	7.5	0.0339	2.6	x = 2.0 ppm
6	7.0	0.0315	2.4	σ = 0.5
7	4.5	0.0195	1.5	σ^2 = 0.25
8	4.5	0.0195	1.5	R.S.D. = $\pm$ 25%
9	7.3	0.0329	2.5	
10	7.0	0.0315	2.4	

Table 10

Daily Variations in the Concentration of Hg in Urine

Date	ppm Hg in Urine	
	Subject 1	Subject 2
10/14/81	1.3	0.4
10/20/81	1.0	
10/30/81	0.5	
11/04/81	0.5	
11/18/81	3.3	
11/30/81	2.3	
01/20/82	1.5	1.7
01/27/82	0.3	0.1
01/29/82	1.6	2.5
02/01/82	0.3	0.2
02/10/82	1.3	0.8
02/15/82	0.6	0.5
03/03/82	0.3	1.5
03/05/82	0.5	1.1
03/10/82	0.4	N.D.
03/17/82	0.3	1.5
03/22/82	0.4	1.0
03/24/82	1.4	0.5
03/29/82	0.5	0.7
03/31/82		0.3
04/02/82	0.1	
04/05/82		0.3
04/19/82		1.6
05/10/82		0.4
05/24/82		0.4
06/02/82		0.5
	$n = 20$	$n = 20$
	$\bar{x} = 0.9$ ppm	$\bar{x} = 0.8$ ppm
	$\sigma = 0.8$	$\sigma = 0.6$

N.D. = none detected (<0.05 ppm Hg)

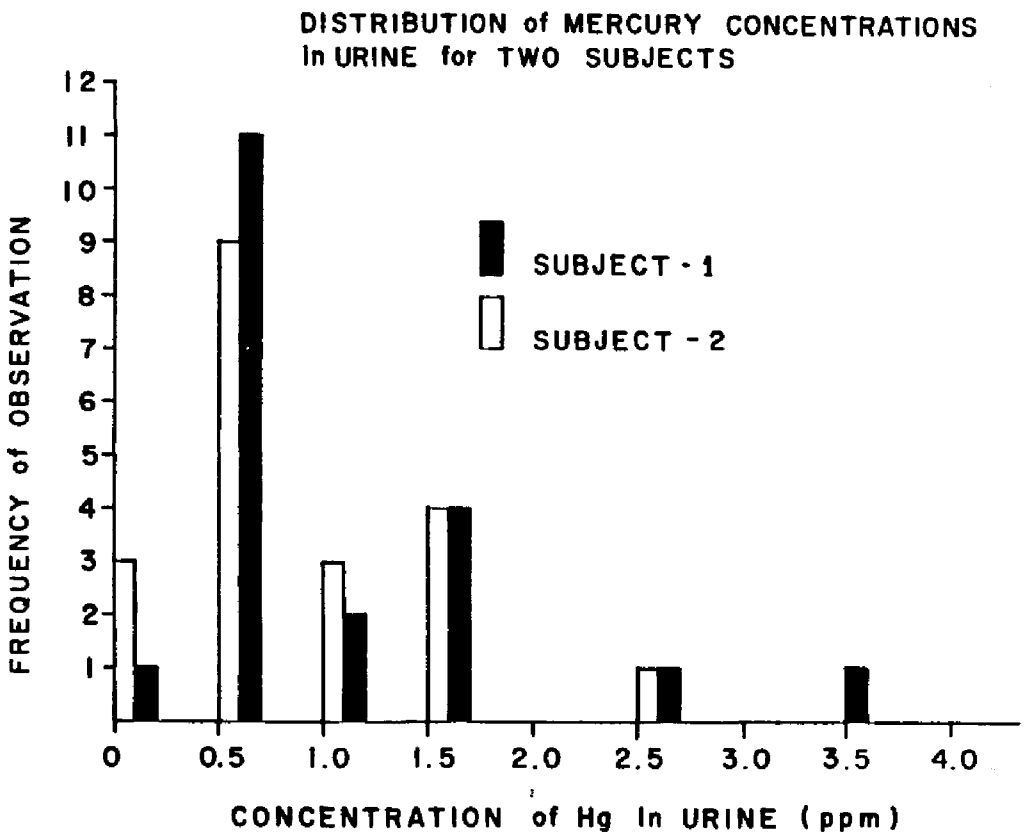


FIGURE 26: DISTRIBUTION OF MERCURY CONCENTRATIONS IN URINE FOR TWO SUBJECTS. THE RANGE OF CONCENTRATIONS COVERED BY THESE DISTRIBUTIONS INDICATED THE WIDE INDIVIDUAL VARIATIONS IN MERCURY LEVELS IN URINE. THE SHAPE OF THE DISTRIBUTIONS IS SIMILAR FOR THE TWO INDIVIDUALS AND THE VARIATIONS APPEAR TO BE RANDOMLY DISTRIBUTED.

on urine mercury levels. The samples were allowed to sit at room temperature in capped polyethylene vials. No preservatives were added. The data collected showed a loss of mercury with time. On the average, 51.5% of the initial mercury concentration was lost.

D. DISCUSSION

1. Advantages of the Method

The use of the described technique for the direct analysis of mercury in urine eliminated many of the errors inherent in other methods. Both positive errors due to contamination and negative errors due to incomplete extraction or volatilization were avoided because no sample pretreatment or preconcentration was necessary.

The efficient atomization achieved by the use of the quartz "T" completely eliminated molecular absorption at the 253.7 nm line. All chemical forms of mercury were broken down to the atomic state and measured, thereby improving the accuracy of the determination in comparison to other methods.

2. Concentration Range for Mercury in Urine

An average concentration of 1.3 ppm Hg was found in the urine of the subjects sampled. This is considerably higher than the 20-50 ppb Hg range for normal urine mercury levels which is reported in the literature. It must be remembered that the limited literature values available were obtained by techniques which have been shown to be inaccurate. The digestion-dithizone extraction method often resulted in incomplete breakdown and

extraction of mercury while the isotope exchange method determined only inorganic mercury. It is believed that the data obtained in this study using the quartz "T" atomizer reflected more accurately the "normal" concentration of mercury in urine.

Although none of the sampled population was occupationally exposed to mercury, one subject worked with elemental and alkylmercury on a limited basis. This subject had the highest urine mercury concentration found in this study, 3.7 ppm Hg.

3. Variations in the Concentration of Mercury in Urine of An Individual

The concentration of mercury in the urine of an individual was found to vary on a day-to-day basis, as had been reported in previous literature. It can be seen from Table 10 that the amount of this variation was not very different for the two subjects studied. The average concentration for Subject 1 was 0.9 ± 0.8 ppm; that for Subject 2 was 0.8 ± 0.6 ppm. The average daily variation for these subjects was 82%.

It was not surprising that the daily excretion of mercury in urine varied. Diet, fluid intake, exercise, and the levels of many other compounds in the body may affect the daily balance of mercury. Certainly, fluid intake directly affected the concentration of mercury found in the urine by altering the amount of fluid passed through the kidneys. Correcting the mercury concentration for the urine specific gravity or for some other urine component such as creatinine can be done to reduce the variation, but it has been reported to be ineffective.[10]

4. Precision of the Method

The relative precision of urine analysis by this method was calculated to be about 28%. The relative precision of the method for aqueous standards was calculated to be about 16%. The inhomogeneity of urine compared to aqueous standards is believed to be responsible for the poorer precision of the urine analysis. Only a 1 or 2 μL aliquot of sample was taken for analysis. A slight change, for example, in the solids content, of so small a volume could result in a large difference in the measured concentration. Urine samples also seemed to soak into the carbon disks more readily than aqueous standards. This often resulted in changes in peak shape and height.

5. Changes in Concentration of Mercury in Urine on Storage

Storage of any sample to be analyzed for mercury can result in loss of mercury through volatilization and adsorption, as was pointed out in the General Introduction. Storage of biological samples is a particular problem because bacterial activity can volatilize mercury from the sample.[147] It is clear from the data in Table 11 that even one or two days storage at room temperature can cause significant loss of urine mercury. Although room temperature storage of biological samples is not a common practice in clinical labs, specimens are often shipped to labs by mail or air freight. Evaporation of the wet or dry ice packing is a common occurrence. Samples which are received at room temperature should not be analyzed for mercury.

Table 11

Change in Concentration of Hg in Urine with Time

	Days from Collection				
	0	1	2	5	
Sample #	ppm Hg Found				% Change in Hg conc.
1	0.5	0.2			-60
2	1.5		0.6		-60
3	0.3		0.3		0
4	0.6		0.3		-50
5	0.5		0.3		-40
6	1.6			0.6	-63
7	2.5			1.6	-36

Average Loss = 51.5%

6. Estimated Daily Excretion of Mercury through Urine

The average daily excretion of mercury through urine could be estimated based on the data obtained in this study. The average concentration of mercury in the urine of the sampled population was 1.3 ppm. If a normal volume of urine excreted in 24 hours is assumed to be 1500 mL,[145,146] then the average amount of mercury excreted per day would be approximately 2 mg.

E. CONCLUSIONS AND SUMMARY

1. The use of the quartz "T" atomizer and the sample introduction techniques described was effective in the direct analysis of mercury in urine. Efficient reduction of the background absorption eliminated the need for pretreatment involving oxidation or digestion of the sample. The method was sufficiently sensitive to allow analysis of 1 μL of urine without preconcentration.

2. The average urinary mercury concentration for individuals who were not occupationally exposed to mercury was found to be 1.3 $\pm$ 0.9 ppm. The relative precision of this method was approximately 28%.

3. Urinary mercury concentration in an individual varied on a day-to-day basis. The average daily variation was 82%.

4. The average daily excretion of mercury through urine was estimated to be 2 mg.

CHAPTER 3

THE DIRECT DETERMINATION OF MERCURY IN SWEAT

A. INTRODUCTION

Excretion of trace metals in perspiration, or sweat, can be of importance in the balance of elements in the human body. The loss of essential trace elements through perspiration can be a factor in nutrition studies and excretion of toxic metals would play a role in toxicity studies. It has been suggested[162] that such excretion is a means of removing non-essential components from the body. Iodine, bromine, silver, mercury and other pharmacological substances administered can be discharged in this manner.[163] In 1928, Lomholt[134] found small amounts of mercury in the perspiration of patients injected with mercuric ion as a treatment for syphilis. The secretion by the sweat glands is far greater than the secretions of most other larger glands; for example, typical sweat glands weighing a total of 40 g secrete 3-10 kg of fluid/day compared to typical saliva glands, which weigh 70 g and secrete 1.5 kg saliva/day.[163] Since a portion of normal human perspiration is insensible and does not involve the sweat glands,[163] loss of trace substances in sweat is possibly more significant than generally believed. Little research has been published on the concentration of metals in human perspiration.

1. Characteristics of Human Perspiration

a. Sensible and Insensible Sweating

Normal human perspiration consists of sensible and insensible sweating.[163] Insensible sweating is that elimination of moisture which occurs without the knowledge or awareness of the subject. Sensible sweating is that which can be felt by the subject.

Insensible sweating includes elimination through respiration and through the skin. The mechanisms for these pathways differ, but both are known to be influenced by factors such as body size, metabolic activity, and atmospheric conditions.[163] Total insensible perspiration has been estimated[162] at 6-19 g/hr, depending on the volume respired and the humidity.

Cutaneous insensible perspiration varies with the region of the body.[2] The palms and soles of the feet are 5-20 times more active in this form of sweating than the general body surface. One type of insensible sweating, thermal sweating, occurs over the general body surface with the exception of the palms and soles. The other type, mental (or emotional) sweating occurs primarily at the palms and soles. The composition of sweat is thought to vary depending on the region of the body producing it.[162,163]

b. Mechanisms of Sweat Excretion

Sensible and insensible perspiration are known to differ in their mechanism of excretion. Insensible perspiration does not involve the sweat glands to any great extent. In the skin, the epidermis is supplied with moisture from the blood

vessels. Insensible perspiration consists of the evaporation of this moisture through the skin.

In contrast, sensible perspiration is always a result of the secretion of the sweat glands.[163] It is known that sensible perspiration is excreted from two types of sweat glands. The more numerous type, the eccrine glands, are distributed all over the body and secrete a dilute fluid with low concentrations of many substances. The apocrine glands are found in association with hair follicles in a few restricted areas of the body. The secretion of the apocrine glands contains many organic substances which vary with different glands and with individuals. Apocrine glands are activated by intense pain or fear.[163]

c. Composition of Sweat

Eccrine sweat, a clear aqueous solution, is generally 99.0-99.5% water and 0.5-1.0% solids which are about half organic and half inorganic.[146] Some common constituents of normal human sweat are listed in Table 12. Most of what is known about the chemical composition of sweat comes from analysis of sweat secreted onto the skin. The composition of sweat as it is formed in the coil of the gland remains largely unknown, but it is believed to be isotonic with plasma.[146,163] Variable re-absorption has been postulated to occur in the gland duct because excreted sweat contains substances whose concentrations are higher than, and lower than, that in plasma.[146,163] The composition of insensible sweat is not known, but could be expected to differ

Table 12

Selected Constituents of Normal Human Sweat[146,163]

Constituent	Concentration
Solids	1.17 - 1.59%
Calcium	1.0 - 24 mg/100 mL
Chloride	320 mg/100 mL
Copper	0.06 mg/L
Iron	0.027 mg/100 mL
Magnesium	0.004 - 0.286 mg/100 mL
Manganese	0.006 mg/100 mL
Phosphorus	0.009 - 0.043 mg/100 mL
Potassium	21 - 126 mg/100 mL
Sodium	24 - 312 mg/100 mL
Sulfur	0.7 - 7.4 mg/100 mL
Zinc	93 µg/100 mL
Bicarbonate	1.6 - 18.6 vol%
Sugar, as glucose	0 - 3 mg/100 mL
Lactic acid	285 - 338 mg/100 mL
Creatinine	0.1 - 1.3 mg/100 mL
Urea	12 - 57 mg/100 mL
Amino acid N	1.1 - 10.2 mg/100 mL

pH - 3.8 - 8.2

specific gravity = 1.001 - 1.006

from that of sensible sweat.

Sweat excreted during the initial stages of perspiration contains both secretions from the sweat glands and contaminants from skin tissue. Sweat obtained during more profuse perspiration was thought to contain only the excretion from the sweat glands.[163]

d. Average Amount of Perspiration

The average amount of perspiration has been estimated to be 3.0-3.3 kg/day for a 65 kg (165 lb) man at 29°C. Sensible perspiration was believed to account for approximately 2.3 kg of this total. Many factors influence the amount of perspiration excreted by an individual, including atmospheric conditions, metabolic rate and body size.

2. Problems in the Analysis of Sweat

The analysis of sweat is made difficult by several factors. Collection of a representative sample is difficult because the composition of sweat appears to vary with the region of the body where it is produced.[162] Collection of a sample must be done without contamination from the skin or the collection vessel. The concentration of many substances in sweat changes with a change in the rate of secretion of sweat. Some substances, such as chloride, increase in concentration as the rate of sweating increases, while others, such as glucose, are inversely related to the rate of sweat secretion.[163] Prevention of evaporative loss of water from the collected sample is also very

difficult.[163] All of these factors undoubtedly contribute to the wide concentration ranges reported in the literature for even major sweat components such as sodium, potassium and chloride.

Several methods for the collection of sweat samples have been reported. A total-body washdown procedure has been used,[162-165] in which subjects were exposed to a controlled-temperature chamber for a period of time, after which the whole body was washed to collect sweat solutes. The amount of sweat was calculated by measuring the net loss in body weight during the collection period. Others have collected sweat in a polyethylene bag attached to a subject's forearm.[166,167] Micropunctures of the sweat gland duct also have been used to collect samples.[163]

Molecular background absorption by salts and organic components of sweat is a problem in spectroscopic analysis of sweat, as it is in urine analysis. Loss of mercury through volatilization during storage or sample digestion and contamination of the sample through added reagents are also problems that must be considered.

3. Common Methods for the Analysis of Sweat

Few examples of sweat analyses can be found in the literature. Lomholt reported[134] in 1928 that small amounts of mercury were found in the sweat of syphilitic patients undergoing mercury injections. The analytical method used was not described, but was probably a gravimetric technique such as the Reinsch test.

Other older papers[163,164,167] reported the determination of metals such as iron, zinc, silver and calcium in sweat by various wet chemical methods and colorimetric procedures. X-ray fluorescence has been used[168] to determine bromine in sweat.

Atomic absorption spectroscopy has been used to determine metals in sweat.[165,166] Cohn and Emmett[165] determined cadmium in sweat by AAS. Asayama, et al.[166] reported a high correlation among the concentrations of 9 metals in sweat. The levels of most metals in sweat were higher than those in urine. Mercury was not among the metals studied.

4. Goals of This Study

In view of the limited data available on the concentration of mercury in human perspiration, it was thought to be appropriate to investigate this excretory mode. Since levels of several metals were found to be higher in sweat than in urine, sweat could play an important role in the excretion of toxic metals from the body.

Carbon bed atomic absorption spectroscopy using the quartz "T" atomizer could be applied easily to the analysis of sweat samples. In conjunction with sample introduction on carbon disks or by direct injection, the described apparatus provided a rapid and accurate means of analysis for a difficult biological matrix.

B. EXPERIMENTAL

1. Equipment

The equipment used in the analysis of sweat samples was

identical to that previously described for the analysis of urine and water.

2. Procedure

a. Sampling Techniques

Sweat samples were collected under supervision in a sauna at the LSU Field House, from male volunteers who were members of the university population. Individuals sampled had undergone an exercise program and then showered before entering the sauna. Sweat samples were collected only after profuse sweating had begun in order to avoid collection of skin contaminants. Collection was made by catching drops of sweat from the nose or forehead in a previously cleaned polyethylene vial. No contact was made between the vial and the skin itself. The average volume of sweat collected was approximately 2 mL over a 10 minute period.

Collection of most samples was made in the late afternoon. In most cases, urine samples were collected from each subject within minutes of the collection of sweat specimens. It thus was possible to compare the mercury content of these two excretory fluids collected at essentially the same time under the same conditions for each individual.

b. Analytical Procedure

All samples were analyzed within three hours of collection. The sample introduction techniques used were described previously. In the direct injection technique, 2 µL

aliquots of sweat were injected with the modified Drummond microdispenser onto the hot carbon bed. In the carbon disk technique, 1 μL of sweat was placed on a heat-cleaned carbon disk by means of a Hamilton syringe. The sample-laden disk was dropped onto the heated carbon bed. The concentration of mercury was determined by measuring the absorption of either the 184.9 or 253.7 nm mercury resonance line. This signal represented both absorption due to mercury and non-specific absorption due to molecular background. Background correction was accomplished by measuring the absorption by the sample of the deuterium lamp signal at either 184.9 or 253.7 nm. The difference between the two signals was a measure of the mercury absorption. The absorption of blank carbon disks was routinely recorded and subtracted from the sample signal when necessary.

c. Calibration Procedures

Calibration was accomplished using aqueous mercuric chloride standards, exactly as described for water analysis. The linear range extended from 0.1 to 10 ng Hg at 253.7 nm.

The method of standard additions was tried on two sweat samples from 2 different subjects; in both cases, the slope was the same as the slope of the aqueous calibration curve. Therefore, sample absorption signals were compared directly to the aqueous standards, and the standard addition method was not used.

As with the urine samples, peaks from direct injection of sweat with the Drummond microdispenser were taller, sharper and

narrower than peaks from carbon disk introduction. Therefore, sample peak height had to be compared to standards which had been introduced into the atomizer in the same manner as the samples.

C. RESULTS

1. Use of the 184.9 nm Resonance Line

Preliminary sweat analyses were carried out at 184.9 nm to take advantage of the increased sensitivity of this line. Introduction of 1 μL sweat samples on carbon disks gave rise to 30-70% absorption of the hollow cathode lamp signal and 10-20% absorption of the deuterium lamp signal. This large background absorption signal was undesirable, particularly since the AAS system was only a single beam system and the background correction could not be made simultaneously with the atomic signal measurement. Analysis of sweat samples at 253.7 nm was tried and typical absorption signals of 2-10% were observed, with no background absorption signal. Therefore, mercury determinations in sweat were carried out at 253.7 nm.

2. Concentration Range for Mercury in the Sweat of Non-Occupationally Exposed Individuals

Sweat samples were collected from twenty-one members of the university population who were not occupationally exposed to mercury. All subjects were male and most were between 40 and 60 years of age. Seven of the subjects were sampled more than once.

The results of the sweat analyses are presented in Table 13. The average mercury concentration for 21 individuals was 0.5 ppm

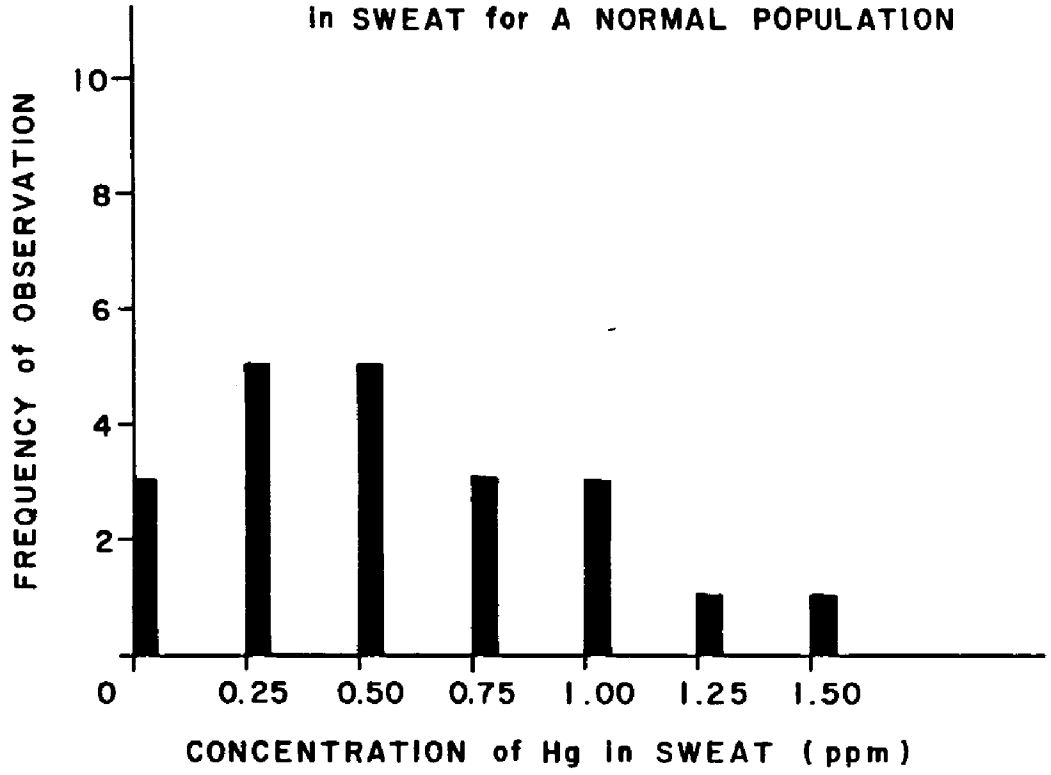
Table 13

Concentration of Mercury in the Sweat of Normal Adults

Subject	ppm Hg in Sweat
1	0.8
2	0.7
3	0.4
4	1.0
5	0.9
6	0.2
7	0.3
8	0.6
9	0.1
10	0.4
11	0.8
12	1.2
13	1.4
14	0.4
15	0.6
17	0.1
19	0.2
20	none detected
21	0.8

$n = 21$
$x = 0.5$
$\sigma = 0.4$
$\sigma^2 = 0.14$
range = <0.1 - 1.4 ppm Hg

FIGURE 27: DISTRIBUTION of MERCURY CONCENTRATION in SWEAT for A NORMAL POPULATION



Hg; the range of values extended from <0.1 ppm to 1.4 ppm. The distribution of mercury concentrations in sweat is illustrated in Figure 27. The concentrations exhibited a fairly normal distribution pattern.

3. Variations in the Mercury Concentration in the Sweat of An Individual

Sweat from two individuals was analyzed repeatedly over an eight month period in order to follow day-to-day changes in the mercury concentration. It was observed that the mercury concentration in sweat did indeed fluctuate on a daily basis, just as did mercury concentrations in urine. The results of this study are presented in Table 14. The distribution of these values is illustrated in Figure 28. The distributions again appear to be reasonably normal. The mean concentration found in the sweat of Subject 1 was 0.8 ± 0.6 ppm Hg, with a range of <0.1 - 2.5 ppm Hg and an average daily variation of 75%; Subject 2 had a mean concentration of 0.7 ± 0.5 ppm, a range of <0.1 - 2.2 ppm and an average daily variation of 71%.

The "standard deviation" has been calculated here as a measure of the amount of variation found in mercury concentrations on a day-to-day basis. It must be emphasized that a different sample was analyzed to obtain each of the reported values. Therefore, the "standard deviation" calculated above is not a measure of precision or of true deviation of the procedure. This value serves only as an indication of the range of mercury

Table 14

Daily Variations in Concentration of Hg in Sweat

Date Mo/Day/Yr	Subject 1 ppm Hg	Subject 2 ppm Hg	Date Mo/Day/Y	Subject 1 ppm Hg	Subject 2 ppm Hg
07/06/81	1.5	1.1	01/20/82	1.1	1.4
07/08/81	1.0	1.0	01/22/82	1.4	NS
07/13/81	ND	1.0	01/27/82	0.1	0.2
07/14/81	0.7	0.7	01/29/82	1.0	1.1
07/20/81	1.0	1.2	02/01/82	0.6	0.6
07/27/81	1.8	0.6	02/10/82	0.6	0.6
09/30/81	2.5	NS	02/12/82	0.3	0.3
10/06/81	0.8	0.8	02/15/82	0.6	0.5[a]
10/07/81	ND	0.2	02/17/82	0.5	0.3
10/14/81	0.9	0.4	02/19/82	0.3	0.3
10/29/81	1.2	NS	03/03/82	0.8	0.1
10/30/81	0.2	0.2	03/05/82	0.1	ND[a]
11/02/81	1.4	1.4	03/10/82	ND	0.5
11/04/81	0.7	0.7	03/17/82	0.7	0.5
11/18/81	1.7	1.4	03/22/82	0.3	0.3
11/30/81	1.6[a]	2.0	03/24/82	0.3	0.3
12/02/81	1.8	1.6	03/29/82	0.2	0.5
12/11/81	2.3	1.4	04/02/82	0.1	NS
12/14/81	0.8	0.7	04/05/82	0.3	0.3
12/16/81	0.8	2.2	04/19/82	NS	0.5
			05/24/82	NS	0.5
			06/02/82	NS	0.6
				n = 39	n = 38
				x = 0.8	x = 0.7
				σ = 0.6	σ = 0.5
				σ^2 = 0.40	σ^2 = 0.25

a - sweat sample turned pink after collection
ND - none detected (<0.1 ppm)
NS - no sample collected

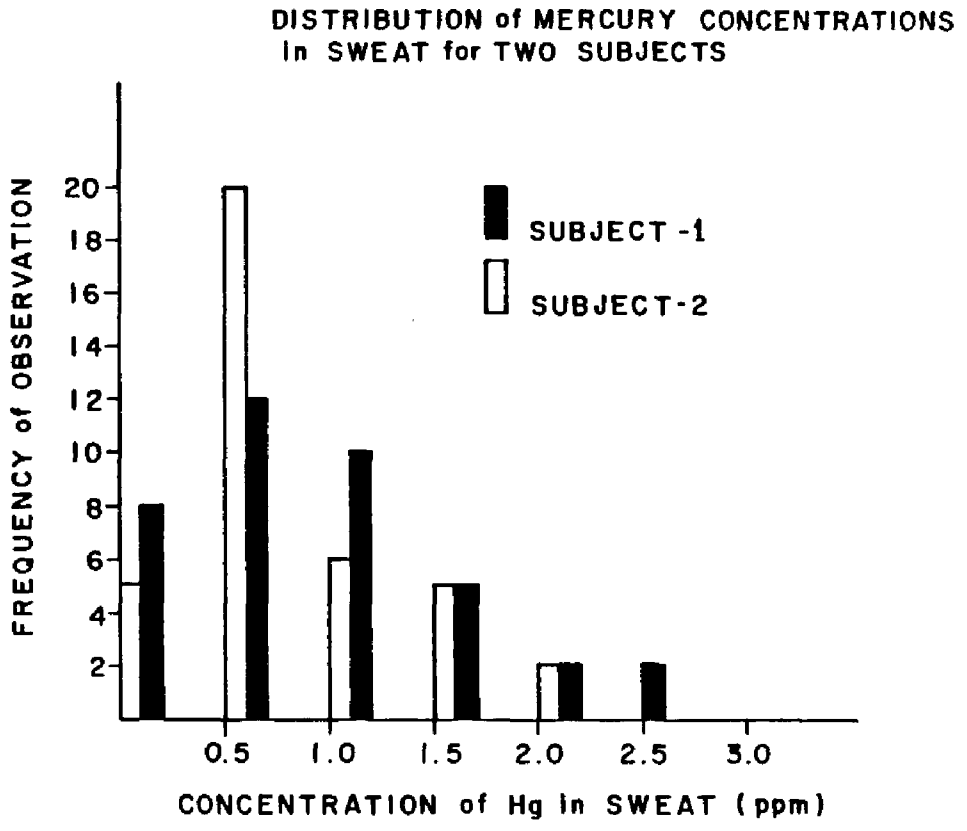


FIGURE 28: DISTRIBUTION OF MERCURY CONCENTRATIONS IN SWEAT FOR TWO INDIVIDUALS. THE RANGE OF CONCENTRATIONS INDICATED THE INDIVIDUAL VARIATION IN MERCURY LEVELS IN SWEAT FROM DAY-TO-DAY. THE SHAPE OF THE DISTRIBUTIONS IS SIMILAR FOR THE TWO SUBJECTS STUDIED AND THE VARIATIONS APPEAR TO BE RANDOMLY DISTRIBUTED.

concentrations in sweat and of the distribution within that range.

On three occasions, marked with a superscript "a" in Table 14, sweat samples in closed polyethylene vials turned pink shortly after collection. This pink color penetrated the polyethylene vial as well as the sample, and could not be removed by overnight soaking in 50% HNO_3, by washing with soap and water or with acetone. The color did not seem to have any effect on the mercury concentration in the sweat sample.

4. Precision of the Analytical Method

A single sweat sample was analyzed repeatedly in order to determine the precision of the method. Sixteen injections with the Drummond micropipette (2 μL aliquots) gave a mercury concentration of 0.9 ± 0.2 ppm (mean + σ). This is equivalent to a relative standard deviation of 22%. Twenty injections of the same sweat sample on carbon disks (1 μL aliquots) gave a relative standard deviation of 18%. The average total absorption signal for direct injection of 2 μL of sweat was 7.6% absorption, with no absorption of the deuterium lamp signal.

5. Comparison of the Mercury Concentrations in Urine and in Sweat

Urine samples were collected in conjunction with sweat samples from the two individuals who were sampled repeatedly for variability studies. Both sweat and urine samples were analyzed within three hours of collection. The concentration of mercury in these two excretory fluids could be compared for each subject:

these data are presented in Table 15.

The ratio of mercury in sweat to mercury in urine fluctuated on a daily basis for each individual. For Subject 1, the average sweat to urine ratio was 0.9 with a range of <0.2 to 2.7; for Subject 2, the average sweat to urine ratio was 1.1 with a range of <0.1 - 5.0. The range of observed values for the mercury concentration in sweat to mercury concentration in urine ratio was much greater for Subject 2 than for Subject 1. A graphical presentation of the correlation data for Subjects 1 and 2 can be found in Figures 29 and 30. Correlation coefficients of 0.815 and 0.451 were calculated for subjects 1 and 2, respectively.

6. Effect of Storage on Mercury Concentrations in Sweat

Several sweat samples were kept at room temperature for a number of days after collection and were analyzed at different intervals to observe the effect of storage on the mercury concentration. The data are presented in Table 16. No change was noted after one day of storage but up to 100% of the original mercury present was lost after 2-5 days. In one case, a 20% increase in mercury was noted after 2 days of storage. This could be due to leaching out of mercury from the polyethylene vial or evaporation of the sweat matrix with a resulting increase in apparent mercury concentration.

D. DISCUSSION

1. General Considerations in the Analysis of Sweat for Mercury

The use of the quartz "T" atomizer and either the direct

Table 15

Comparison of the Concentration of Mercury in Urine and Sweat of Two Individuals (Repeated Sampling)

[Hg] in sweat (ppm) / [Hg] in urine (ppm)		
Date	Subject 1	Subject 2
10/14/81	0.9/1.3 = 0.7	0.4/0.4 = 1.0
10/29/81	1.2/1.0 = 1.2	NS
10/30/81	0.2/0.5 = 0.4	NS
11/04/81	0.7/0.5 = 1.4	NS
11/18/81	1.7/3.3 = 0.5	NS
11/30/81	1.6/2.3 = 0.7	NS
01/20/82	1/6/2.3 = 0.7	1/4/1.7 = 0.8
01/27/82	0.1/0.3 = 0.3	0.2/0.1 = 2.0
01/29/82	1.0/1.6 = 0.6	1/1/2.5 = 0.4
02/01/82	0.6/0.3 = 2.0	0.6/0.2 = 3.0
02/10/82	0.6/1.3 = 0.5	0.6/0.8 = 0.8
02/15/82	0.6/0.6 = 1.0	0.5/0.5 = 1.0
03/03/82	0.8/0.3 = 0.2	0.1/1.5 = 0.1
03/05/82	0.1/0.5 = 0.2	ND/1.1 =<0.1
03/10/82	ND/0.4 =<0.2	0.5/ND =>5.0
03/17/82	0.7/0.3 = 2.3	0.5/1.5 = 0.3
03/22/82	0.3/0.4 = 0.8	0.3/0.5 = 0.6
03/24/82	0.3/1.1 = 0.3	0.6/1.0 = 0.6
04/02/82	0.1/0.1 = 1.0	N.S.
04/05/82	NS	0.3/0.3 = 1.0
04/19/82	NS	0.5/1.6 = 0.3
05/24/82	NS	0.5/0.4 = 1.2
06/02/82	NS	0.6/0.5 = 1.2
	n = 20	n = 18
	x = 0.9	x = 1.1
	σ = 0.7	σ = 1.2
	range = <0.2-2.7	range = <0.1->5.0

ND = none detected (<0.1 ppm) NS = no sample

FIGURE 29: CORRELATION BETWEEN MERCURY CONCENTRATIONS IN SWEAT AND IN URINE. NO GOOD CORRELATION EXISTED BETWEEN THE MERCURY CONCENTRATIONS IN SWEAT AND THOSE IN URINE FOR SUBJECT 1 (CORRELATION COEFFICIENT= 0.815). THE CIRCLED DOT INDICATES OVERLAP OF TWO DATA POINTS.

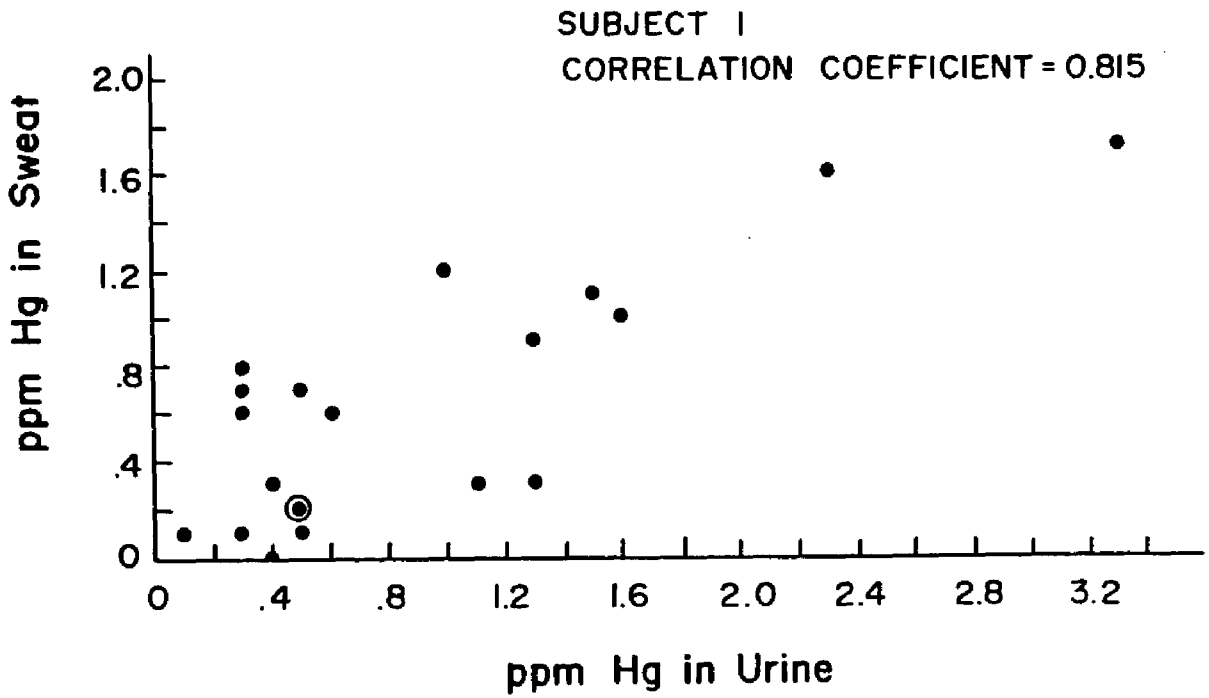


FIGURE 30: CORRELATION BETWEEN MERCURY CONCENTRATIONS IN SWEAT AND IN URINE. NO CORRELATION EXISTED BETWEEN MERCURY CONCENTRATIONS IN SWEAT AND MERCURY CONCENTRATIONS IN URINE FOR SUBJECT 2 (CORRELATION COEFFICIENT = 0.451).

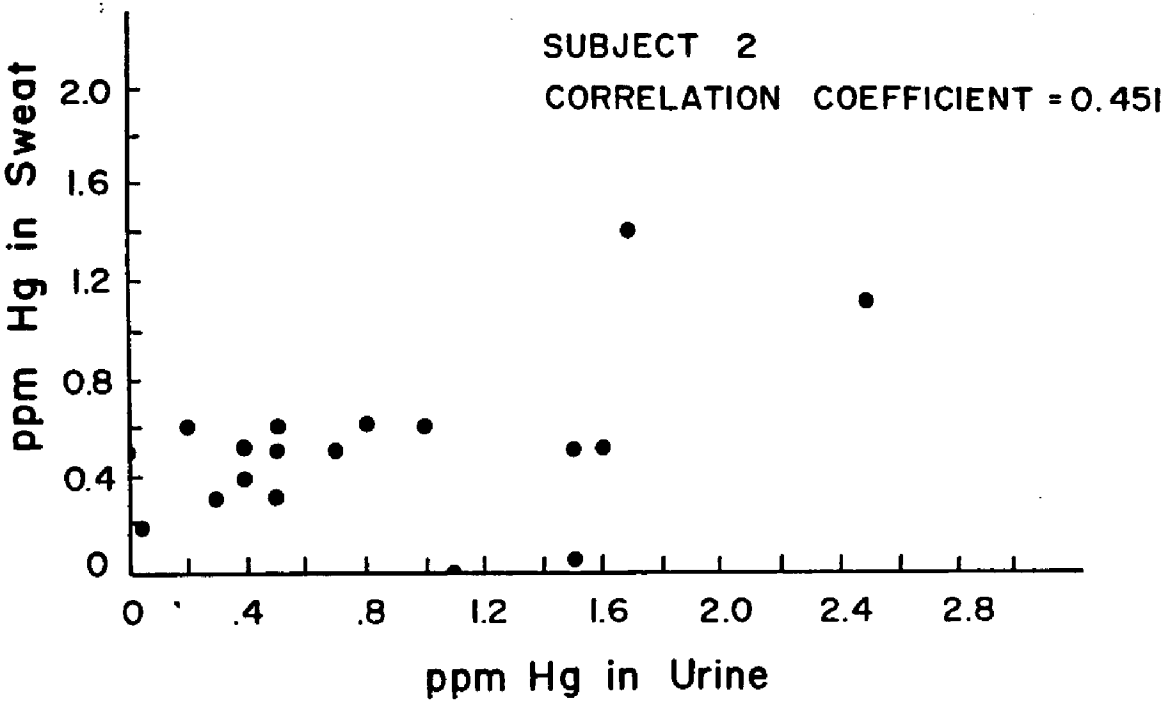


Table 16

Effect of Storage on Mercury Concentrations in Sweat

Sample	Days from Collection 0	1	2	3	5	% Change in Mercury Concentration
	ppm Hg					
1	0.2	0.2				0
2	0.8		ND			-100
3	0.1		ND			-100
4	0.6		0.3			- 50
5	0.5		0.6			+ 20
6	0.3			0.3		0
7	0.3			0.2		- 33
8	1.0				0.6	- 40
9	1.1				0.5	- 45
10	0.3				ND	-100
11	0.3				ND	-100

ND = none detected (<0.1 ppm)

injection or carbon disk introduction technique allowed the direct determination of mercury in sweat by providing efficient breakdown of the matrix. This eliminated background absorbance and thereby eliminated the need for preliminary digestion of the sample. Sensitivity was sufficient to permit analysis without preconcentration of the mercury in the sample. The described technique therefore avoided the errors associated with sample pretreatment and greatly simplified the analytical procedure.

The precision of the analytical method was determined to be approximately 20% at the 0.9 ppm level. The precision was considered to be acceptable taking into account the extremely small volume of sample injected and the lack of a double beam atomic absorption system. Drop hang-up was a slight problem, even with the modified Drummond microdispenser and any volume error in a 1 or 2 μL aliquot would result in a large concentration error. A double-beam optical system might have improved the precision by providing simultaneous background correction.

2. Concentration Range for Mercury in the Sweat of Non-Occupationally Exposed Individuals

A fairly normal distribution of mercury in sweat was found in the population analyzed in this study, with a mean of 0.5 ppm Hg.

Only limited data were available in the literature regarding the level of mercury in perspiration. Kuno[163] reported that

mercury could be discharged in sweat, but gave no concentrations or references. Consolazio et al.[169] estimated that 0.9 μg Hg was lost per day through sweat. If the normal volume of human sweat is taken[163] to be 3 L/day, 0.9 μg Hg/day corresponds to a concentration of 0.3 ppb Hg.

The values obtained for mercury concentrations in sweat by the method described herein are believed to be reasonable and accurate because of the direct collection of the sweat samples and the direct analysis technique used.

3. Estimated Daily Excretion of Mercury Through Perspiration

The sweat samples collected for this study were considered to represent sensible perspiration. Under the conditions of sample collection (in a sauna), it may be considered that maximum sweating took place. It has been estimated[163] that the maximum perspiration rate is approximately 1.5 kg/hr. Using the mean value of 0.5 ppm Hg found, under these conditions an individual will excrete approximately 0.75 mg Hg/hr. This rate of sweating cannot be continued for more than a few hours, however.

A normal volume of approximately 3 L of sweat is excreted per day by an adult male weighing approximately 65 kg. Therefore, using the mean value of 0.5 ppm Hg found in this study, it can be estimated that 1.5 mg Hg/day will be excreted through sweat. The 3 liters of sweat excreted per day includes an estimated 1 liter of insensible perspiration. The composition of insensible

perspiration is thought to differ from sensible perspiration since both sweat components are produced by different mechanisms.[163] The mercury concentration may or may not be equal in the two components. In addition, it is known that the rate of sweating affects the concentration of some excreted substances[163]; chloride concentration increases with increased rate of sweating, for example, while glucose and amino acid concentrations are inversely related to the rate of sweating. Both of these factors must be kept in mind when considering the data obtained in this study.

It seems, based on the data reported here, that excretion of mercury through perspiration is a definite mode of elimination of the metal.

4. Variations in the Mercury Concentration in Sweat for An Individual

The mercury concentration in sweat was found to vary for an individual sampled on different days. As Table 14 showed, the amount of variation was slightly different for the two subjects studied. The mercury concentration range in the sweat of Subject 1 had a variance of 0.40 while that of Subject 2 had a variance of 0.25.

It was not surprising that the concentration of mercury in sweat varied on a daily basis. Many factors could influence this variation, including diet, exercise, weather, and the efficiency of the sweat glands. Although external variations, for example

those due to atmospheric differences, were minimized by the collection of samples always under the same conditions, normal daily variations in the factors mentioned above could affect total perspiration and therefore daily mercury excretion.

5. Comparison of the Mercury Concentrations in Sweat and in Urine

It was of interest to discover whether a correlation existed between the concentration of mercury in sweat and urine, since both fluids are modes of excretion in man. As is illustrated in Figures 29 and 30, no good correlation was evident between the concentration of mercury in these fluids for the two individuals studied. Subject 1 had a correlation coefficient of 0.815 while Subject 2 had a correlation coefficient of 0.451. It would appear that a better correlation between mercury levels in urine and sweat existed for Subject 1 than Subject 2. This could be due to excretion of different chemical forms of mercury by the individuals. If the chemical form of mercury excreted in sweat is the same as that excreted in urine, it might be expected that a correlation would exist between the two levels. On the other hand, urine and sweat are excreted by two very different mechanisms for two very different purposes. Excretion of sweat is a temperature-regulating mechanism, while excretion of urine is a means of waste removal. Moreover, the half-life of mercury in the kidney is much longer that the half-life of mercury in other parts of the body (except the brain), with the result that mercury is

concentrated in the kidney. Therefore, it might not be reasonable to expect a correlation between urine and sweat mercury levels.

If different chemical forms of mercury were excreted in urine and sweat, or if the chemical form of excreted mercury varied on an individual basis, it might be expected that analysis by the method of standard additions would show differences in the slopes of the calibration curves for different media or individuals. As was discussed earlier in this chapter, and in the chapter on urine analysis, the slopes of the standard addition calibration curves did not vary between individuals or matrices and were in fact equal to the slope of the aqueous mercuric chloride calibration curve. This does not rule out differences in chemical form, but could reflect only the efficiency of the quartz "T" atomizer. If all mercury-containing species are completely atomized, no difference in slope would be seen.

Determination of the exact chemical form of mercury in sweat and urine was a highly desirable goal. Discussion of the techniques used for speciation attempts and the results obtained may be found in Part II of this disseration.

6. Effect of Storage on Mercury Concentrations in Sweat

As can be seen in Table 16, significant quantities of mercury are lost from sweat on storage at room temperature in capped polyethylene vials. An average of 50% of the original mercury is lost over a 2 - 5 day storage period. The change in mercury concentration varied from sample to sample; for example,

samples held for two days showed changes in mercury concentration ranging from +20% to -100%. Samples held for five days showed changes of -40% to -100%.

The mechanisms of loss of mercury from sweat are most probably the same as those for urine: volatilization by bacterial action, vaporization out of the vial, and adsorption onto the container walls. Each of these factors can contribute to individual variation in the loss of mercury from sweat. The number and kind of bacteria present in sweat vary from subject to subject, and therefore, bacterial volatilization of mercury might vary from one subject to another. Vaporization losses from solution will depend on the volatility (i.e., chemical form) of the mercury present and on the permeability of the vial, which would be controlled by wall thickness, age of the vial, density of the polyethylene and tightness of fit of the cap. Adsorption would vary with the active sites on the vial wall, which are probably dependent on the cleaning process used, physical differences in the wall surface from vial to vial and similar factors.

E. CONCLUSIONS AND SUMMARY

1. The use of the quartz "T" atomizer together with direct injection or sample introduction on a carbon disk was a simple and reliable technique for the direct analysis of sweat samples. Efficient breakdown of the sample eliminated background absorption, so that pretreatment of the sample was not required.

The method was sufficiently sensitive so that preconcentration of the sample was unnecessary.

2. The average concentration of mercury in the sweat of the sampled population was found to be 0.5 ppm. The subjects were members of the university community and were not considered to be occupationally exposed to mercury. The relative standard deviation of the method was approximately 20%.

3. The concentration of mercury in the sweat of an individual varied on a daily basis. This variation was approximately 71-75%.

4. The approximate rate of excretion of mercury through perspiration under conditions of profuse sweating was calculated to be 0.75 mg/hr.

5. If it was assumed that the composition of insensible sweat was the same as sensible sweat, the average total mercury excretion through sweat was estimated to be 1.5 mg/day.

6. No general correlation was observed between the daily fluctuations in urine and sweat mercury levels.

7. The slopes of the standard addition calibration curves for urine and sweat were similar for the subjects studied. This indicated that the matrix effects were similar for the two types of samples and that the mercury excreted through these fluids may be in the same chemical form.

8. Mercury in sweat is lost on storage at room temperature, with as much as 100% of the original mercury present lost over a 2-5 day period.

CHAPTER 4

THE DIRECT DETERMINATION OF MERCURY IN WHOLE BLOOD AND SERUM

A. INTRODUCTION

1. The Use of Whole Blood and Serum as Biological Indicators of Mercury Exposure

Despite the widespread use of mercury concentrations in blood in the evaluation of human exposure to mercury, little work has been done to establish a "normal" range of concentration. Published experimental results have indicated that the correlation between mercury exposure and concentration in blood is not firmly established. The correlation depended strongly on the chemical form of the mercury exposure, among other things. Whole blood and its components, red blood cells (RBCs), plasma and serum, have been studied in attempts to elucidate the metabolism of mercury in the human body.

Whole blood is a very complicated sample matrix.[145] It consists of cellular elements (red and white blood cells and platelets), proteins, carbohydrates, lipids and inorganic ions. The specific gravity of whole blood is 1.06 and the viscosity is about 6 times that of water. The major ions present in blood include sodium,potassium, magnesium, calcium, chloride, phosphate, bicarbonate and sulfate. Table 17 presents some selected constitutents of whole human blood.[145]

Red blood cells, or erythrocytes, have a membrane composed of

Table 17

Selected Constituents of Human Blood Plasma[145]

Organic Constituents	Normal Range (mg/100 mL)
Protein	5700 - 8000
Amino Acids	35 - 65
Cystine	0.8 - 5.0
Glutamic Acid	0.4 - 4.4
Glutamine	4.5 - 10
Methiomine	0.2 - 1.0
Urea	20 - 30
Glucose	65 - 90
Polysaccharides	70 - 105
Lipids	285 - 675
Cholesterol	130 - 260

Inorganic Constituents	Normal Range (meq/L)
Sodium	132 - 150
Potassium	38 - 5.4
Calcium	4.5 - 5.6
Magnesium	1.6 - 2.2
Iron	0.009 - 0.032
Copper	0.001 - 0.002
Chloride	110 - 110
Bicarbonate	24 - 30
Phosphate	1.6 - 2.7
Sulfate	0.7 - 1.5
Iodine	0.0006 - 0.001

49% protein, 44% lipid and 7% carbohydrate, and approximately 6 nm thick. Potassium is the chief cation in RBCs while chloride is the chief anion. Other major constituents of RBCs are sodium, calcium, magnesium, bicarbonate, hemoglobin and 2,3-diphosphoglycerate. Red blood cells can be separated from whole blood by addition of an anticoagulant and centrifugation.

Plasma is the clear yellowish supernatant obtained from centrifuged uncoagulated whole blood. All the cellular elements have been removed, but plasma still contains fibrinogen. Serum is the clear, yellowish supernatant obtained from clotted blood; all cellular elements and fibrinogen have been removed. Solutes constitute 10% of the volume of plasma. These solutes are 7% protein and 0.9% inorganic salts, with the remainder being non-protein organic material.

Whole blood is capable of absorbing mercury vapor and rapidly oxidizing it to mercuric mercury. Magos[81] studied the *in vitro* uptake of mercury vapor in plasma and red blood cells. Only 8% of the mercury retained by the blood was in the elemental form 5 minutes after exposure. Since the total circulation time in man is about 22 seconds, a large part of any elemental mercury taken up by the blood would be in the elemental form when circulating blood enters brain vessels. It has been proposed[81] that red blood cells serve as accumulators and generators of Hg°, capable of interconversion of Hg° and Hg^{2+}. Release of easily diffusible Hg° from these cells would be responsible for the

greater penetration of mercury into the brain after vapor exposure than after exposure to other forms of mercury. Clarkson et al.[170] found that mercury vapor was taken up faster by hemoglobin solutions and RBCs than by plasma. These results were confirmed by Berlin et al.,[171] who observed 67-84% of mercury in blood bound to RBCs after Hg° exposure compared to 25-31% bound to cells after intravenous injection of mercuric ion. They postulated that non-polar mercury vapor might dissolve in the lipid structures of the erythrocytes.

Inorganic mercury is absorbed into the plasma proteins.[172] Due to the rapidity of oxidation, most mercury in the blood after mercury vapor exposure is expected to be in the mercuric form. Mercuric ion has been shown to be equally distributed between the RBCs and the plasma at equilibrium. At least part of the inorganic mercury in erythrocytes exposed to mercuric chloride in vitro was found in a protein fraction migrating like hemoglobin on paper electrophoresis.[170] Farvar and Cember[173] showed that albumin and globulin fractions of plasma contained mercury. After exposure to inorganic mercury, its concentration in blood was high, but diminished rapidly with time.[10] No useful correlation has been found between concentrations of mercury in blood and exposure to mercury vapor or inorganic mercury compounds.

Exposure to organomercury compounds resulted in a different distribution of mercury in blood than that found after inorganic

mercury exposure. Alkyl mercury compounds have a much higher affinity for red blood cells than for plasma.[174,175] Aberg et al.[174] found about ten times more mercury in RBCs than in plasma after oral administration of methylmercury to human volunteers. Berglund and Berlin[175] reported that at least 90% of the methylmercury found in the blood of several animal species was bound to the red blood cells. The fraction of methylmercury in the plasma varied according to species: 10% in man and rabbits, 4.5% in rats, for example. Takeda et al.[176] showed that mercury in blood after administration of ethylmercury salts was present almost exclusively as ethylmercury bound to a considerable degree to the hemoglobin in red cells. Exposure to monomethylmercury resulted in high blood levels for a considerable time after exposure. The biological half-life of methylmercury in blood has been estimated by radiotracer studies to be 50-70 days.[137,177,178] Blood was found to contain 5-10% of the methylmercury. Since methylmercury is only slowly eliminated and the concentration ratio between mercury in RBCs and plasma is approximately equal to 10 (compared to a ratio of 1 for inorganic mercury exposure), blood mercury levels can provide a good indicator of methylmercury exposure.

The relationship of mercury levels in blood to other organomercury exposure is not clear. Ostlund[80] found low levels of mercury in blood after administration of dimethylmercury to mice. The dimethylmercury was readily transported to fat

deposits and did not remain in the blood. Aberg et al.[174] stated that arylmercury compounds were largely bound to red blood cells, but other authors[175] have found an even distribution between red blood cells and plasma after exposure to these compounds.

It has been shown[10] that mercury concentration ratios between blood and brain and between blood and kidney changed with time after an exposure and during a series of exposures. Therefore, mercury concentrations in blood are not useful as indices of accumulation of mercury in critical organs, especially after exposure to inorganic mercury. Mercury levels in blood reflect mainly recent exposure. On a group basis there is a reasonably good correlation between recent mercury exposure and blood levels.[142] However, blood concentrations, particularly high concentration ratios between mercury in cells and plasma, are the best indication of exposure to methylmercury.[10]

Mercury levels in serum have not been used extensively to evaluate exposure to mercury,[179] but the mercury levels should not differ significantly from those found in plasma.

2. "Normal" Levels of Mercury in Blood

Data on mercury levels in the blood of a "normal," that is, non-occupationally exposed, adult population are scarce. An international study was conducted by the World Health Organization in 1964.[23] Whole blood samples were collected in 15 countries and were analyzed in one laboratory. The dithizone-extraction,

cold vapor-atomic absorption method of Jacobs et al.[22] was used. Eight hundred and twelve samples of whole blood were analyzed. Seventy-seven percent of all samples were found to have a mercury concentration of less than 5 ppb (the analytical zero of the method). Ninety-five percent of all samples had a mercury concentration of less than 30 ppb. Levels greater than 100 ppb were found in 1.5% of the samples. A study of 382 adults not occupationally exposed to mercury was conducted by Smith et al.[142] Blood samples were analyzed by the same dithizone-AAS method used in the study discussed above. Mercury concentrations of less than 0.01 ppm were found in 69.3% of the samples; concentrations of 0.01-0.05 ppm were found in 30.7% of the samples.

The dithizone-AAS method of Jacobs et al.[22] which was used to obtain the above data will be discussed below in the section on methods of analysis for blood samples, but it should be emphasized here that the values obtained by this method are almost certainly too low. The method only required partial oxidation of the blood matrix. It has been shown[143,180] that incomplete oxidation of organic material results in incomplete extraction of mercury by dithizone.

Suzuki et al.[181] determined mercury concentrations in the blood cells and plasma of nine pregnant women with no history of mercury exposure. They found 22.9 ng Hg/ml in the red cells and 12.4 ng Hg/ml in plasma. Data from Sweden[8] on mercury levels in blood

cells and plasma have found 2-5 ng Hg/g blood cells and 1.3 ng Hg/g plasma in non-exposed subjects.

Exposure to methylmercury through ingestion of contaminated fish resulted in increased levels of mercury in blood. Concentrations of 7-248 ppb Hg in whole blood have been reported following such exposures.[182-184] Mercury concentrations of 2-20 ppb in red blood cells following methylmercury exposures have been reported with values of only 2-3 ppb found in plasma.[2,10] Workers in a chlorine plant who were occupationally exposed to elemental mercury vapor were studied by Smith et al.[142] Almost half of the 567 workers had concentrations in whole blood of >0.10 ppm Hg, which was at least ten times higher than non-exposed controls. Concentrations in serum of 1.9-2.9 ppm Hg were found in five subjects who had ingested methylmercury contaminated pork.[179]

In general, wide variations have been reported in concentrations of mercury in blood, plasma and serum. Many studies did not give adequate information regarding the population sampled or the method of analysis to allow meaningful conclusions to be drawn. Many of the techniques used for analysis undoubtedly gave low results because of inefficient extraction procedures. All that can be said is that low estimates of mercury concentrations in a non-exposed population are 0.03-0.05 ppm in whole blood and 0.01 ppm in plasma.

3. Analytical Problems in the Determination of Mercury in Blood and Serum

Problems in the analysis of blood and serum begin with collection of the sample. Needles and syringes can contaminate the sample if they are not properly cleaned. Trace metals in the blood sample can be absorbed onto the wall of the syringe needle and thereby lost from solution. Blood samples are difficult to store. An anticoagulant, such as disodium EDTA or potassium oxalate, must be added to whole blood samples. Anticoagulants are often chelating agents, which prevent clotting by binding calcium ion. Such agents are difficult to purify of trace metal contaminants and can add to the metal concentration in a sample. Blood collection tubes and rubber stoppers can contaminate a sample with excess metals or adsorb metals from solution. Care must be taken to avoid loss of volatile mercury compounds from samples during storage. Use of a chelating agent as an anticoagulant can help by binding some chemical forms of mercury and preventing volatization and adsorption. Samples should be refrigerated for short-term storage and frozen for long-term storage.

The variable and dense matrix of blood causes difficulties in analysis. Blood contains high concentrations of inorganic ions, as can be seen in Table 17. The principal cations are sodium and potassium; the predominant anion is chloride. Blood also contains numerous and diverse organic compounds such as lipids, carbohy-

drate and proteins of a wide range of molecular weights.[145] These components can cause analytical interferences when the matrix is not completely decomposed. High molecular background absorption can be a problem in atomic spectroscopic methods, since it limits both the sensitivity and the accuracy of the method.[185] The variable and complex matrix makes it impractical, if not impossible, to create standard solutions representative of the sample. This significantly complicates calibration procedures.

The viscosity of blood samples makes it difficult to obtain reproducible, representative sample aliquots using micropipettes.

Most current methods of analysis include pretreatment of the blood sample to destroy the organic material. Wet digestion of the sample with concentrated mineral acid and an oxidizing agent such as potassium permanganate is commonly employed. The dangers of sample contamination from added reagents and loss of mercury on heating sample digests are significant. Dry ashing procedures should not be used when mercury concentrations are to be measured. Vaporization losses prior to the atomization stage result in serious negative errors in the determination of mercury by graphite furnace-AAS.

4. Common Methods for the Determination of Mercury in Whole Blood and Serum

Most official or recommended procedures[2,180] for the determination of mercury in biological samples have been wet oxidation-dithizone extraction colorimetric methods. An example

of this type of procedure for the analysis of blood samples was reported by Mikettukova and Kacl.[186] Blood was digested with H_2SO_4/HNO_3 in a flask with a reflux condenser. Potassium permanganate was added to finish the oxidation. The digest was treated with urea to remove nitrous gases and with EDTA to mask interferences. Mercury was extracted as the dithizonate into chloroform and measured by colorimetry. The detection limit was 0.2 ppm Hg.

The most current methods for the determination of mercury in blood are neutron activation analysis and atomic absorption spectroscopy.

Neutron activation analysis[2,183,184] has been used as a highly specific and sensitive method for the analysis of blood for mercury. Samples were usually sealed in quartz or polyethylene vials and irradiated with neutrons to convert ^{196}Hg to ^{197}Hg. The ^{197}Hg, a radioactive isotope with a 65-hr half-life, was identified with a multichannel analyzer and Ge(Li) detector.[182] Post-irradiation separation of mercury was necessary because of the low concentrations present and the interferences due to radioactivities produced by other components. Mercury can be lost from samples by volatilization during irradiation.[33] Significant disadvantages to this method included excessive equipment cost relative to other methods, the need for a nearby neutron source and an inability to determine the chemical form of mercury in samples.[25]

Flame atomic absorption spectroscopy has been used to determine mercury in whole blood and serum. A variety of sample pretreatment steps have been used. Berman[56] precipitated protein from samples with trichloroacetic acid and adjusted the pH of the supernatant to 2.8-3.5. Mercury was extracted with APDC into MIBK. The MIBK layer was aspirated into an oxidizing flame. A sensitivity of 0.1 ppm was reported, with 95-105% recovery from spiked samples. Later work by Schulert et al.[158] showed that precipitation with trichloroacetic acid and extraction with APDC/MIBK only recovered 2-10% of added radiotracer mercury. Curley et al.[179] analyzed serum by flame-AAS. Samples weighing 5.4-36.5 g were refluxed for one and a half hours with HCl and HNO_3. The pH was adjusted to 2.5-3.5 with NaOH and the mercury was chelated and extracted with three portions of APDC in MIBK. The volume of MIBK was reduced from 150 mL to 4-20 mL and solutions were aspirated into an air-H_2 flame using a total consumption burner. A mercury-containing guanidine compound was used as a standard to simulate protein bound mercury; standards were carried through the digestion and extraction procedure. A recovery of 98-100% at the 5-50 ppm level was reported.

Cold vapor-AAS techniques have been shown to be much more sensitive than flame AAS methods and are used almost exclusively for the routine determination of mercury in biological samples. Jacobs and coworkers[22] partially digested blood samples (0.1 - 1.0 g) with H_2SO_4 and $KMnO_4$ over low heat. Mercury was

extracted from these partial digests with dithizone in chloroform. The extract was placed in an ignition tube and the $CHCl_3$ evaporated. More intense heating evolved Hg° vapor, which was carried by an air stream through several cold traps into a gas absorption cell. The mercury vapor was determined by AAS. An analysis required 3 hours. A sensitivity of 0.001-0.01 ppm was reported, with recovery of 56-106% at the nanogram level. It has been shown,[143,180] however, that mercury is not completely extracted by dithizone from partially digested organic materials. Therefore, this method probably resulted in negative errors.

Lidums and Ulfvarson[159] combusted blood samples (20-200 mg) in O_2 and passed the combustion gases through a series of traps and scrubbers to a gold filter. Mercury vapor was trapped on the gold and was later released into an AAS gas cell by heating the filter. Analysis required 30 minutes and had a sensitivity of 1 ppb and a precision of 10%.

Many variations of the reduction-aeration CV-AAS technique have been used to analyze blood.[181,188] $SnCl_2$ is used to reduce Hg^{2+} in oxidized samples to Hg°. The solution is aerated and the mercury is carried into a gas absorption cell. Sensitivities of about 10 ppb are usual.

The methods described above are used to determine total mercury concentrations. Specific organic mercury compounds have been determined in blood by the gas chromatographic technique of Westoo.[84] Samples (10-50 g) were homogenized, acidified,

extracted into benzene, back-extracted into aqueous cysteine acetate, reacidified and re-extracted into benzene prior to GC analysis.

5. Need for an Improved Analytical Technique

None of the analytical methods described above provided a true direct determination of mercury in blood or serum, for which no sample pretreatment or concentration steps were used. Such a direct method was desirable because it would eliminate both contamination due to added reagents and losses due to volatilization. A direct method with sufficient sensitivity would eliminate negative errors due to incomplete extraction or inefficient trapping of mercury from the sample. The speed and simplicity of the analysis would be significantly increased.

The use of the quartz "T" atomizer made the direct analysis of blood and serum possible. There were several advantages in the use of this technique. The matrix was effectively reduced to CO and H_2, which reduced molecular background absorption. Mercury was efficiently atomized, eliminating chemical interferences. No sample was lost because all of the sample components were drawn through the bed and the light path. The absorption signal was constantly monitored. The efficient atomization and long light path provided sufficient sensitivity so that preconcentration of mercury in the sample was unnecessary. In addition, the quartz "T" atomizer permitted use of the more sensitive 184.9 nm resonance line as well as the more commonly employed 253.7 nm line.

B. EXPERIMENTAL

1. Equipment

The equipment used in the analysis of blood and serum samples was identical to that described in Chapter 1. The 253.7 nm and 184.9 nm resonance lines were each used; operating conditions were the same as those previously employed.

2. Analytical Procedure

a. Sampling Techniques

Whole blood and serum samples were obtained from the LSU Student Health Service laboratory. Samples were collected from LSU students by Health Service personnel, using disposable sterile syringes and glass Vacutainer blood collection tubes. Dipotassium EDTA was present in the Vacutainers to prevent coagulation of the whole blood samples. Serum was obtained from centrifugation of blood samples which had been collected in plain glass Vacutainers (no anticoagulant present). Samples were stored at 4°C. Samples were analyzed on the day after collection.

b. Development of a Sample Introduction Technique

i. Direct Injection

Sample introduction into the atomizer was attempted by direct injection of 5 µL aliquots of whole blood and serum with an Eppendorf micropipette. The sample was injected onto a hot (1450°C) carbon bed. This method gave very poor reproducibility. Successive aliquots gave widely varying absorption signals, as can be seen in Figure 31. Absorption traces varied from sharp spikes to broad, tailing peaks. This was not

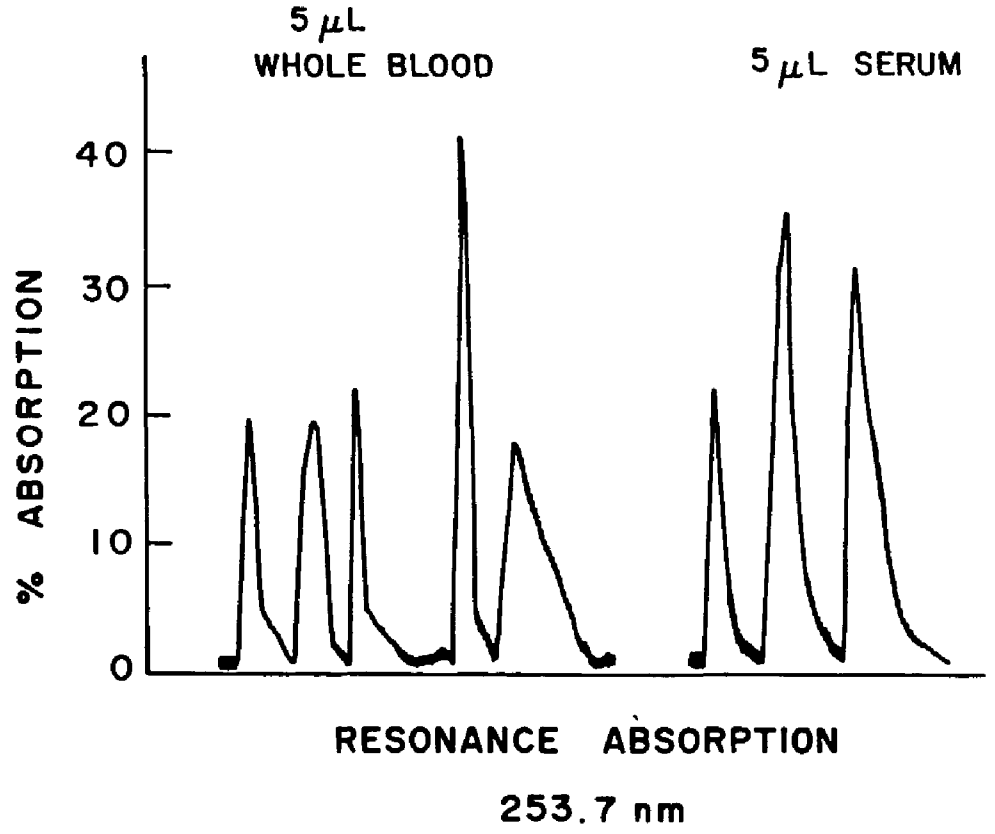


FIGURE 31: ABSORPTION SIGNALS FROM WHOLE BLOOD AND SERUM. BROAD, IRREGULAR PEAKS WERE OBTAINED ON DIRECT INJECTION OF 5 μL ALIQUOTS WITH AN EPPENDORF MICROPIPETTE. PEAKS WERE UNSUITABLE FOR QUANTITATION BY MEASUREMENT OF PEAK HEIGHT DUE TO THEIR IRREGULAR SHAPE.

desirable because it required measurement of the area under the peak rather than the peak height. It was difficult to estimate the area under asymmetrically-shaped peaks. The poor reproducibility was probably due to the viscosity of whole blood and serum. It was difficult to completely eject all of the sample from the pipette. At times, heat from the carbon bed caused coagulation of blood on the pipette tip if the sample was not ejected rapidly. Whole blood samples especially were not homogeneous. Often, small clots would be left in the pipette tip or would block the tip during sample ejection. For these reasons, a consistent amount of sample was not always injected to the atomizer. Injection of 5 µL aliquots often caused visible disruption of the carbon bed surface, with carbon dust being dislodged into the air above the bed. After some injections, an explosion occurred as the sample burned. These observations indicated that part of the reason for poor reproducibility was a difference in reaction of the sample at the carbon surface. Explosions caused carbon dust to be blown into the light path, which scattered radiation. This was confirmed by the observations of high molecular background absorption and a pile of carbon dust in the light path.

ii. Filter Paper Disk Method

Small filter paper disks had been used for the determination of cadmium in whole blood using the quartz "T" atomizer.[189] Introduction of a 1 µL sample on a filter paper disk had been found to reduce molecular absorption by increasing both the contact time and the contact area of the sample with the

carbon bed. Samples which were introduced on filter paper disks appeared to burn more evenly than samples which were directly injected onto the carbon bed.

Filter paper disks were prepared by cutting Whatman #41 ashless filter paper into disks of 3 mm diameter with a hole punch. Disks were cleaned by two overnight soakings in 10% HNO_3, using two fresh portions of acid. The disks were placed in a Buchner funnel, rinsed well with distilled, deionized water and partially dried by vacuum-suction. Disks were allowed to dry completely in a clean-air environment.

A 1 µL aliquot of blood or serum was placed on a filter paper disk with a Hamilton microliter syringe. The disk was dropped into the atomizer with a pair of cleaned stainless steel tweezers. Samples and standards were analyzed in the same manner.

Several problems were encountered with this technique. Acid-cleaned filter paper disks generated widely varying resonance absorption signals of up to 30% absorption at 184.9 nm and up to 10% absorption at 253.7 nm. As can be seen in Figures 32 and 33, this absorption signal was mostly due to mercury in the filter paper disks, because the molecular absorption signals were considerably smaller than the atomic absorption signals. The wide fluctuations in absorption intensity can be seen, especially at 184.9 nm. The variation in absorption signal was probably due to inconsistent cleaning of the disks by the method used. Attempts were made to further clean the disks by heating. A layer of disks was placed on a layer of carbon pieces in the atomizer inner

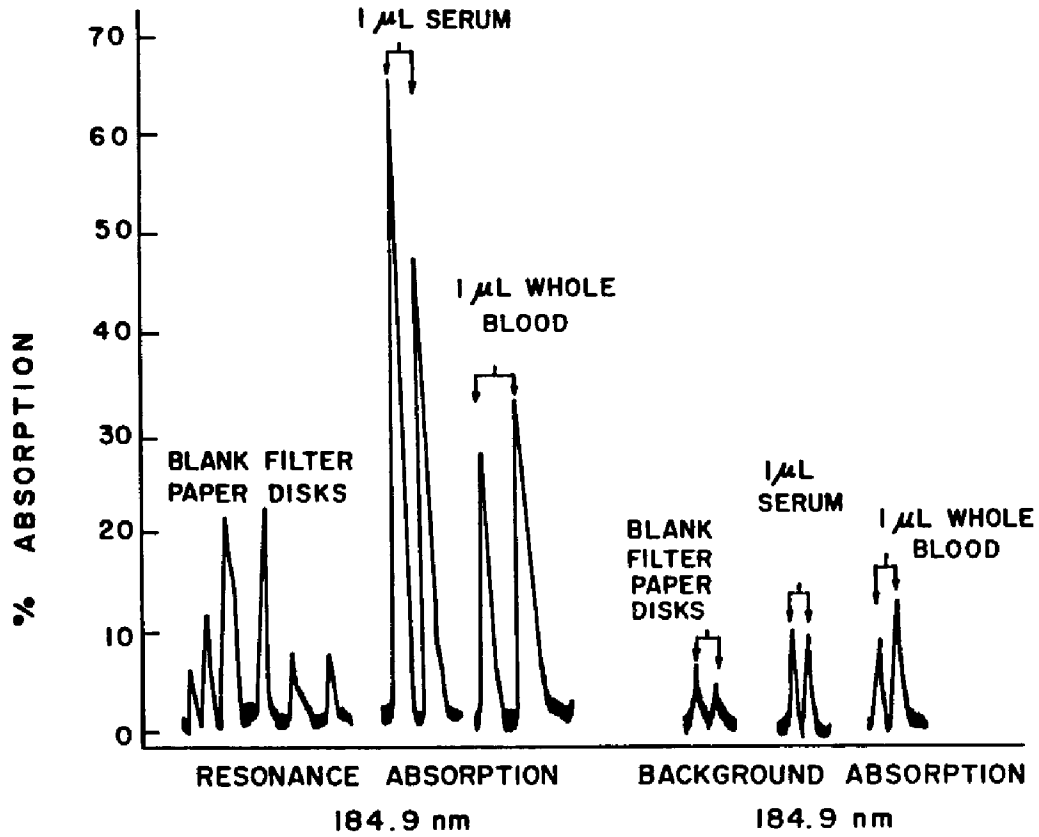
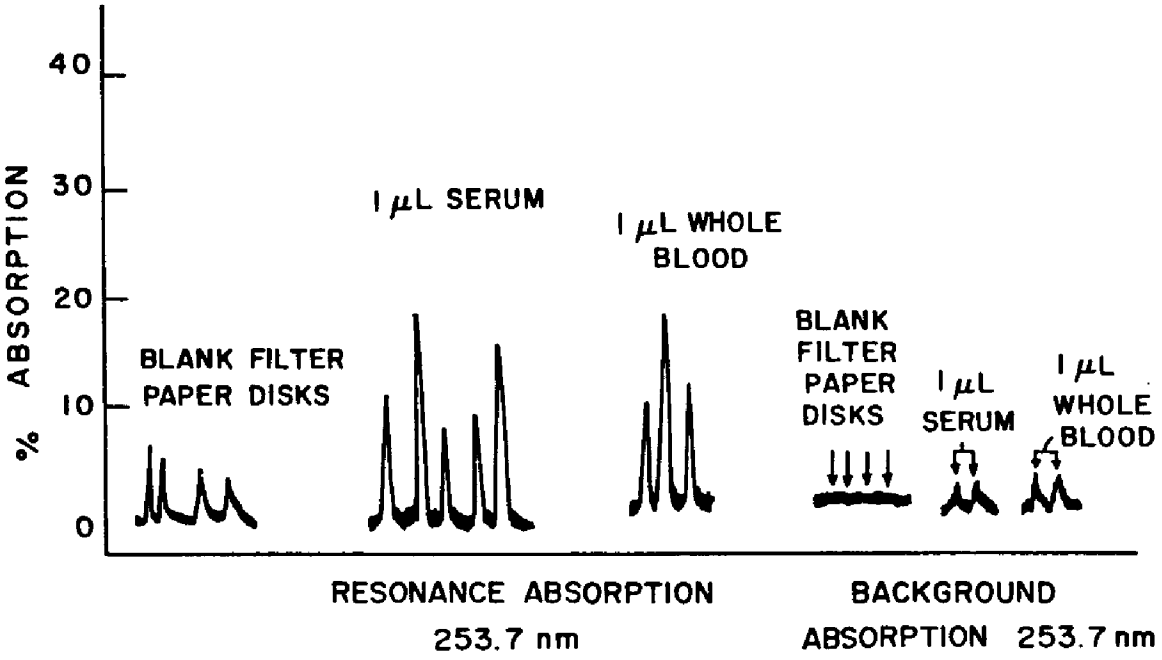


FIGURE 32: ABSORPTION SIGNALS FROM WHOLE BLOOD AND SERUM ON FILTER PAPER DISKS AT 184.9 nm. ACID-CLEANED FILTER PAPER DISKS GAVE VARIABLE ABSORPTION SIGNALS OF 5-25%. TYPICAL ABSORPTION SIGNALS FOR 1 μL ALIQUOTS OF BLOOD AND SERUM ARE SHOWN.

FIGURE 33: ABSORPTION SIGNALS FROM WHOLE BLOOD AND SERUM ON FILTER PAPER DISKS AT 253.7 nm. TYPICAL SIGNALS FROM ACID-CLEANED FILTER PAPER DISKS AND 1 μL ALIQUOTS OF BLOOD AND SERUM ARE SHOWN.



sleeve. The rf generator was not turned on, but the disks were heated by air in the heated light path. Disks were heated for 20 minutes. The absorption due to mercury did decrease but the reproducibility of the signal did not improve because disks were charred to different degrees by the process. This caused a variable loss of mercury.

Another problem with the filter paper disks was that the volume of sample injected was limited to 2 μL. Larger volumes soaked the filter paper and tweezer tips and caused the disks to stick to the tweezers by surface tension.

Typical absorption signals for 1 μL aliquots of whole blood and serum put into the atomizer on filter paper disks were shown in Figures 32 and 33. The lack of reproducibility in the signals can be seen. Molecular background absorption ranged from 10-50% of the total absorption signal.

iii. Carbon Disk Method

Introduction of a sample into the atomizer on a disk of Graphoil pyrolytic-coated graphite was described in detail in Chapter 1. This technique was used successfully for the analysis of blood and serum. Carbon disks cleaned by heating in the hot carbon bed gave no atomic or molecular absorption signal, which was an advantage over the filter paper disk technique. This technique had its own problems, however. The carbon disks became porous on repeated use. Samples which soaked into the disks took a longer time to atomizer and gave broader absorption signals than

those which did not. Absorption signals were slightly broader than those obtained from injection on filter paper disks, but were sufficiently sharp and symmetrical to permit measurement of peak height instead of peak area. Typical absorption signals for blood and serum on carbon disks at 184.9 nm and 253.7 nm are shown in Figures 34 and 35. This method was used to obtain the results presented herein.

iv. Stop-Flow Method

A study was conducted to determine if background absorption could be decreased by stopping the flow through the atomizer during sample introduction. Stopping the flow increased the contact time of the sample with the carbon bed, thereby allowing more complete destruction of the matrix. The procedure involved several steps. The flow through the atomizer cell was discontinued before introducing the sample. The sample was introduced on to the hot carbon bed. The flow remained off for a specific time period; in this case, 15 seconds. Then, flow through the atomizer was resumed and sample components drawn into the light path. Diversion of flow through the cell was accomplished by turning a three way valve located between the atomizer cell and the vacuum pump.

A delay time (time that the flow was off after sample introduction) of 15 seconds was used in the hope that it would be sufficient to break down the matrix without allowing volatilized mercury to escape from the top of the atomizer. The absorption

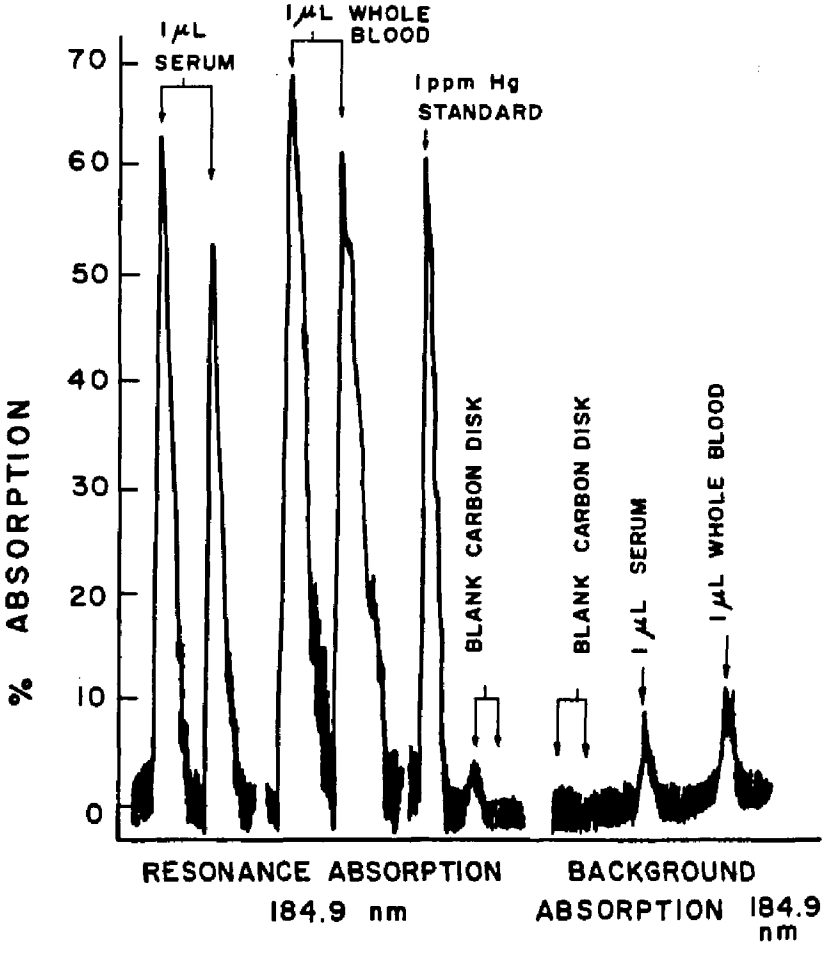
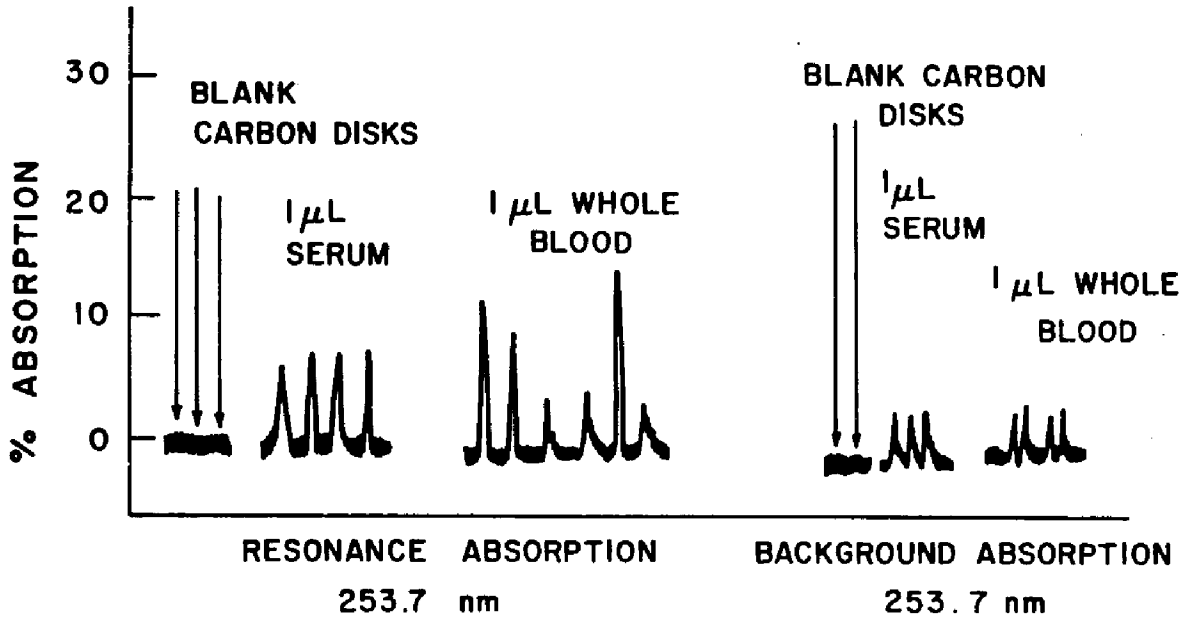


FIGURE 34: ABSORPTION SIGNALS FROM WHOLE BLOOD AND SERUM ON CARBON DISKS AT 184.9 nm .

FIGURE 35: ABSORPTION SIGNALS FROM WHOLE BLOOD AND SERUM ON CARBON DISKS AT 253.7 nm.



data obtained from stop flow analysis of blood and serum on carbon disks are given in Table 18, together with absorption data from the same samples analyzed by the normal procedure (constant flow of 250 mL/min). The signals obtained by the stop-flow method were very irregularly-shaped and irreproducible, especially those of whole blood samples. Background absorption was not significantly reduced and, in some cases, was slightly increased over absorption signals obtained by continuous flow analysis. If flow through the atomizer was resumed too quickly, carbon dust was often pulled into the light path, which gave rise to a sharp absorption spike superimposed on the sample absorption signal.

The greatest difficulty with the technique was in controlling and repeating exactly the conditions for analysis. The time that the flow was discontinued before sample introduction, the delay time, the bed temperature and the flow pattern through the atomizer had to be rigidly controlled and repeated to achieve reproducible results. It was difficult to achieve this control manually. Therefore, the stop-flow technique was not used.

c. Determination of Mercury in Blood and Serum

Whole blood and serum samples were analyzed by placing a 1 μL aliquot on a heat-cleaned carbon disk and dropping it onto the hot carbon bed. A continuous flow of 250 mL/min of air or nitrogen was maintained through the atomizer. Resonance absorption was measured at 184.9 or 253.5 nm with a demountable hollow cathode lamp as the light source. Background absorption was

Table 18

Comparison of Continuous Flow Analysis and Stop-Flow Analysis of Blood and Serum

Sample	Continuous Flow		Stop-Flow	
	Resonance Absorbance	Background Absorbance	Resonance Absorbance	Background Absorbance
1 μL serum	0.0862	0.0000	0.0434	0.0132
on carbon disk	0.0864	0.0088	0.0223	0.0177
(replicate	0.0969	0.0088	0.0362	0.0132
analysis)	0.0706	0.0000	0.0223	0.0177
	$\bar{x}$ = 0.0850	$\bar{x}$ = 0.0044	$\bar{x}$ = 0.0310	$\bar{x}$ = 0.0154
1 μL whole blood	0.0506	0.0088	0.0506	0.0044
on carbon disk	0.0362	0.0044	0.0223	0.0000
(replicate	0.0458	0.0088	0.0757	0.0000
analysis)	0.0292	0.0044	0.0110	0.0088
	$\bar{x}$ = 0.0404	$\bar{x}$ = 0.0066	$\bar{x}$ = 0.0399	$\bar{x}$ = 0.0033

measured at the same wavelength with a deuterium lamp as the light source.

Blank samples for this analysis were prepared by placing a few mL of distilled deionized water into unused blood and serum collection tubes. The tubes were well-shaken and the water analyzed by the carbon disk method. No signals above those generated by distilled deionized water alone were measured. Therefore, no contamination of the sample was caused by the glass tubes, rubber stoppers or anticoagulant.

The sensitivity of the method, defined as the amount of Hg equal to 1% absorption, was 1.0×10^{-10} g Hg at 253.7 nm and 1.5×10^{-11} g Hg at 184.9 nm. The detection limit at 184.9 nm was 0.01 ppm for a 1 µL aliquot.

d. Calibration

Aqueous standards were prepared daily from 1000 ppm Hg (as $HgCl_2$) stock standard solution. Volumetric flasks were pre-equilibrated with mercury standard solutions and were used routinely for preparation of the same standards. Standards of 0.1-10 ppm Hg were used to construct calibration curves. Standards were run at the beginning and end of a series of analyses and several times during the analysis of large numbers of samples. A calibration curve was prepared from standards analyzed in the same manner as the samples.

e. Speciation of Mercury Compounds in Blood and Serum

An attempt was made to identify the number and form of

mercury-containing compounds in blood and serum. A differential volatilization step was used in conjunction with the quartz "T" atomizer. The method and results were discussed in Chapter 7 of this dissertation.

C. RESULTS

1. Precision of the Method

Analysis of whole blood was performed by the carbon disk method at both 184.9 nm and 253.7 nm. Typical absorption traces are shown in Figures 34 and 35 for 1 μL aliquots of both fluids.

Whole blood and serum gave very similar absorption peak heights. At 184.9 nm, the average total signal using the hollow cathode lamp was 40% absorption for a 1 μL aliquot. The average background signal was 15% absorption. At 253.7 nm, the average total signal for a 1 μL aliquot was 8%, and the average background signal was 4% absorption.

The short-term precision of the method was determined by repeated analysis of the same blood or serum sample. Precision was determined at both 184.9 nm and 253.7 nm. The results of a precision study of whole blood are presented in Table 19. The relative standard deviation of the atomic absorption signal for 1 μL of blood at 184.9 nm was 22%; the relative standard deviation of the signal at 253.7 nm was 67%.

2. Concentration Range of Mercury in Whole Blood

Thirty-two whole blood samples were analyzed in this study. The quantitative analyses were performed at 184.9 nm

Table 19

Short Term Precision of Blood Analysis

1 µL of Whole Blood on Carbon Disk	Wavelength Used	
	184.9 nm	253.7 nm
Number of Aliquots	18	19
Average Absorbance	0.0871	0.0096
Standard Deviation	0.0195	0.0065
Variance	0.00038	0.00004
Range of Peak Heights (in % Absorption)	14 - 25%	0 - 45%
Relative Standard Deviation of Absorbance	22%	67%

Table 20

Concentrations of Mercury in Whole Blood

Sample #	ppm Hg	Sample #	ppm Hg
1	0.95	17	0.84
2	1.42	18	0.70
3	N.D.	19	0.55
4	N.D.	20	0.02
5	0.73	21	0.74
6	N.D.	22	0.45
7	N.D.	23	0.50
8	N.D.	24	0.42
9	N.D.	25	0.05
10	N.D.	26	0.90
11	N.D.	27	0.32
12	1.90	28	N.D.
13	1.20	29	0.15
14	0.98	30	0.15
15	0.55	31	0.10
16	0.03	32	0.15

a) N.D. = none detected (<0.01 ppm Hg)
n = 32
$\bar{x}$ = 0.43 ppm
σ = 0.49
σ^2 = 0.23

because of the better precision obtained at this wavelength. The concentrations measured are listed in Table 20. The average concentration was 0.43 ppm Hg. The concentrations ranged from 0.01 to 1.90 ppm. The standard deviation of 0.49 ppm reported in Table 20 was a measure of the distribution of concentrations found in the entire population sampled, not a measure of the deviation of any individual sample.

The distribution of results is shown in Figure 36. Nine samples out of 32 had mercury concentrations below the detection limit of the method (0.01 ppm Hg).

3. Concentration Range of Mercury in Serum

Sixteen serum samples were analyzed in this study. The concentrations measured are given in Table 21. The average concentration was 0.32 ppm Hg. The concentrations ranged from 0.01 ppm to 1.06 ppm. Again, the standard deviation listed in Table 21 was a measure of the concentration range in the population sampled, not a measure of the deviation of an individual sample.

The distribution of mercury concentrations found in serum is illustrated in Figure 36. Half of the samples had concentrations below 0.01 ppm Hg, the detection limit of the method.

D. DISCUSSION

1. Advantages of the Method in the Analysis of Blood and Serum

The use of the quartz "T" atomizer and carbon disk intro-

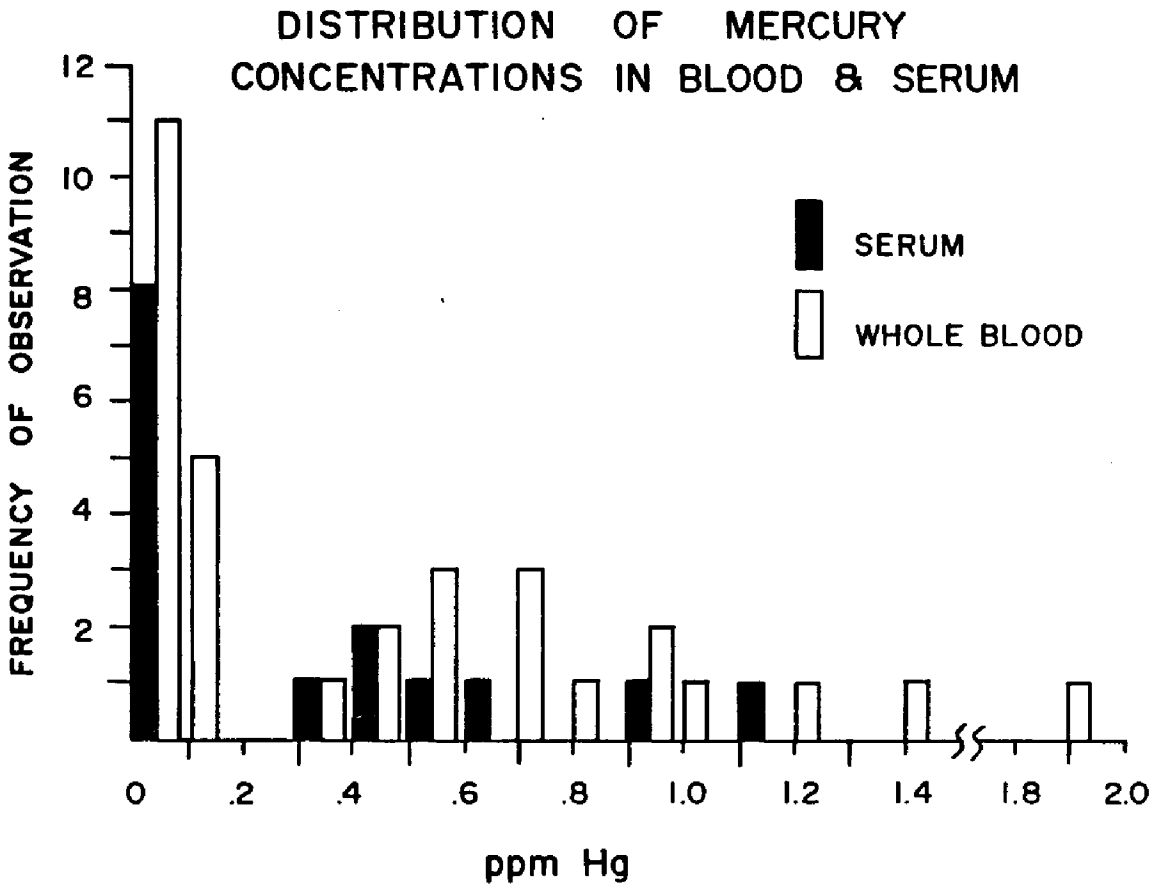


FIGURE 36: DISTRIBUTION OF MERCURY CONCENTRATIONS IN WHOLE BLOOD AND SERUM. CONCENTRATIONS WERE ROUNDED TO THE NEAREST 0.1 ppm Hg.

Table 21

Concentrations of Mercury in Serum

Sample #	ppm Hg	Sample #	ppm Hg
1	0.42	9	0.42
2	0.30	10	N.D.
3	N.D.	11	N.D.
4	N.D.	12	1.06
5	N.D.	13	N.D.
6	0.56	14	0.89
7	0.48	15	N.D.
8	0.99	16	N.D.

a) N.D. = none detected (<0.01 ppm Hg)
$n = 16$
$x = 0.32$ ppm
$\sigma = 0.39$
$\sigma^2 = 0.15$

duction technique provided a simple, rapid, and accurate method for the direct determination of mercury in whole blood and serum. No sample pretreatment was necessary, which eliminated the danger of contamination of the sample by added reagents. Loss of mercury due to sample handling or volatilization was avoided. Samples were efficiently atomized and all sample components were constrained to exit the system through the light path. Thus, all chemical forms of mercury in the samples were measured.

Only microliter quantities of sample were needed, which is a definite advantage in the analysis of blood and serum, since sample quantities are usually limited to a few milliliters.

Use of carbon disks reduced blank absorption to below the detection limit of the method, so that no correction was needed in the absorption of standards and samples. Background absorption and reproducibility were also improved by this technique; however, these parameters still created some problems in the routine application of the method. These problems are discussed below.

2. Weaknesses of the Method

Although absorption due to the organic matrix was reduced by the use of the carbon disk technique, background signals of up to 50% of the total absorption signal were observed. At 184.9 nm, the background absorption was expected due to the formation of CO, H_2 and other small molecules formed upon breakdown of the organic matrix. The stop-flow technique was tried in an attempt to reduce this background, but was not successful. This is

convincing evidence that the background absorption did not result from incomplete degradation of the matrix. Therefore, other changes to the system designed to increase contact time between the sample and carbon bed, such as increasing the size of the carbon bed, would probably not result in a decreased background absorption.

The reproducibility of the absorption signal remained the biggest problem in the analysis of blood and serum by this method. However, this problem was not unique to the carbon disk method, since lack of precision has been a major disadvantage in most of the reported methods for analysis of biological materials.[126] The irreproducibility of absorption signals was believed to be due to two factors, differences in the size of the aliquot delivered and differences in reaction of the sample with the bed.

Any variation in the size of the sample aliquot measured or delivered for analysis would cause a difference in the height of the absorption signal recorded. This was especially a problem due to the small quantities of sample involved (1 or 2 microliters) and the inhomogeneity of blood and serum. Whole blood was more difficult to pipet than serum. The microliter syringe often clogged when transferring whole blood samples and needed frequent cleaning. Serum samples did not clog the syringe, but the viscosity of both fluids made filling and emptying the syringe very difficult.

Variation in the size of the absorption signals could be accounted for by differences in the way samples hit the carbon bed. The bed surface was not homogeneous; some carbon pieces were hotter than others. Dust which accumulated around the edges of the bed in the spaces in between the carbon pieces was cooler than the large carbon pieces. The place of contact between the sample and the bed thus directly affected the speed and efficiency of the atomization. Also, the speed and efficiency of atomization were affected by whether the carbon disk hit the bed edge-on, sample-side down or sample-side up. Samples which landed face-up were heated more slowly than samples which landed face-down on the hot carbon bed.

One disadvantage of this direct determination is the possibility that cobalt, present in Vitamin B_{12} in the blood, could cause a direct spectral interference in analysis at 253.7 nm. This interference was not considered to be important, because cobalt levels in blood are very low and the absorption line is not a resonance line for cobalt. However, the interference would not be present in those AAS techniques which separated mercury from the sample prior to analysis.

3. Precision of the Method

The precision of the carbon disk method for the analysis of blood and serum depended on the wavelength at which the absorption was measured, as can be seen in Table 19. The reason for the poorer precision at 253.7 nm was readily understood by comparing

the size of the absorption signals at the two wavelengths. The signals at 184.9 nm were about seven times the size of the signals at 253.7 nm. The latter signals were less than 5% absorption. It is well known[132] that the error in measurement of a signal increases as the size of the signal decreases. Analyses were carried out at 184.9 nm because of the better precision obtained at this wavelength. The precision could be significantly improved with the use of an automatic background correction system. With the single beam system used in this study, resonance absorption was measured on several aliquots of each sample. Then the deuterium lamp was set up in place of the hollow cathode lamp and background absorption was measured on several aliquots of each sample. The average background signal was subtracted from the average resonance signal. This method was inherently less precise than subtraction of the "real" background contribution to each resonance signal. Conversion of the present system to a double-beam system capable of automatic background correction would have required a complete redesign of the rf induction coils and shielding. Such a major change in the system was not desirable in the middle of a study, but will definitely be considered in future system design changes.

4. Concentrations of Mercury in Whole Blood

The values obtained for concentrations of mercury in whole blood in this study were higher than those reported by other researchers for an unexposed population. This was expected. Many

of the methods used for the determination of mercury in blood have been shown to give low results because of incomplete degradation of the matrix, incomplete extraction of mercury from the sample, or failure to detect all the chemical forms of mercury present in the sample. The direct determination reported here is believed to give a much more accurate estimate of mercury levels in blood than methods requiring oxidation of the sample and separation of the mercury contained therein.

The distribution of concentrations shown in Figure 36 does not appear to be normal, but since one-third of the samples had concentrations of mercury below the detection limit, the appearance of the distribution may be misleading. The center of the distribution may be between 0.01 and 0.10 ppm, with the data points shown in Figure 36 lying to the right of center.

It was unfortunately not possible to characterize the sampled population beyond the fact that it consisted of university students who had visited the Student Health Service. No information on the sex, age or possibility of exposure to mercury was available. It was assumed that the population sampled represented one which was not occupationally exposed to mercury. It should be noted, however, that southern Louisiana is famous for its seafood. Seafood, especially fish, is a well-known source of methylmercury. An exposure to methylmercury through consumption of fish cannot be ruled out.

5. Concentrations of Mercury in Serum

Data are not available on the concentration of mercury in the serum of a "normal" population, but the levels of mercury in plasma may be taken as a guide. The concentrations of mercury in serum found in this study were higher than those reported by other researchers. The mean concentration (0.32 ppm) was lower than the concentrations found in serum following known acute exposure to methylmercury (1.9 - 2.9 ppm).

The sample population was of the same sort as described above, but the serum samples and whole blood samples did not come from the same subjects. It was therefore impossible to compare that ratio of mercury in whole blood to serum to determine the nature of the mercury present.

E. CONCLUSIONS AND SUMMARY

1. The use of the quartz "T" atomizer and the carbon disk technique permitted the direct determination of mercury in whole blood and serum. Only 1 or 2 µL aliquots were required.

2. The use of the quartz "T" atomizer enabled atomic absorption measurements to be made at the more sensitive 184.9 nm resonance line, in the vacuum-ultraviolet region of the spectrum, rather than at the more commonly employed 253.7 nm line.

3. The average concentration of mercury in whole blood from a non-occupationally exposed population was found to be 0.43 ± 0.09 ppm Hg. The range was from <0.01 to 1.90 ppm Hg.

4. The average concentration of mercury in serum from a non-occupationally exposed population was found to be 0.32 $\pm$ 0.07 ppm Hg. The range was from <0.01 to 1.06 ppm Hg.

CHAPTER 5

THE DIRECT DETERMINATION OF MERCURY IN HAIR

A. INTRODUCTION

The specimens most commonly used for routine clinical analyses of mercury concentrations are blood and urine. Unfortunately they tend to reflect only recent exposure. Mercury concentrations in these fluids show a positive correlation with mercury exposure for a group of people[142] (e.g., in an industrial stituation), but show poor correlation with mercury exposure for an individual. Wide diurnal and day-to-day variations in blood and urine have been reported which were independent of exposure.[22] It can be concluded that mercury concentrations in blood and urine neither provide a good evaluation of the accumulation of mercury in tissues[10] nor predict the effects of mercury exposure on an individual.

1. Use of Hair as a Biological Indicator

It has been known for many years that hair has the ability to store quantities of elements which are present in the body, especially elements like lead, arsenic and mercury. Concentrations of heavy metals in hair can provide a time-dependent record of exposure to these metals.[190,191] Hair appears to provide a long-term record of exposure to different chemical forms of mercury via ingestion and topical application.[183,192-194,15,16]

There are many advantages in the use of scalp hair as a biological index of mercury exposure.[195] It appears to provide a time- and concentration-dependent record of exposure. It is relatively stable, fairly homogeneous, easily collected and easily stored. Mercury levels in hair are about two orders of magnitude higher than levels in blood.[10] Hair tissue is a good choice for analysis when a cumulative toxin like mercury is to be determined because it reflects exposure over the time period of growth of the hair.

Hair protein consists of approximately 14% cysteine residues.[145] It is well-known that mercury combines readily with the thiol and disulfide groups present in these residues.[196] It has been concluded that mercury probably exists in hair as an integral part of the hair fiber structure, complexed to the sulfur-containing side chains of the protein.[197]

Interpretation of data on mercury in scalp hair is complicated because a wide variety of factors may influence the concentration. It has been reported that the concentrations of many trace metals, including mercury, increase along the hair shaft from the scalp to the distal end.[195,198,199] It is generally assumed that hair is metabolically inert after it leaves the scalp. Therefore, increased concentrations at the distal end are assumed to arise from external sources. Continuous, but variable, exposure to environmental pollutants and hair treatments can cause external deposition on and diffusion into the hair

matrix of an element like mercury, given both the ion-exchange nature of hair and the strong affinity of mercury for sulfur groups. A question remains as to the nature of the bond between externally-applied mercury and the hair shaft, but the available data indicate that washing does not significantly affect the mercury concentration in hair. Mercury levels in the hair of a non-occupationally exposed population may reflect the ambient mercury levels in the environment. A number of researchers have determined such mercury levels and some reported values for "normal" mercury levels in hair are presented in Table 22. Most studies indicated that average mercury concentrations ranged from 2-9 ppm, with the distribution skewed toward lower concentrations. As can be seen from the data in Table 22, the range of concentrations found within a given study often varies widely.

Mercury concentrations in hair have been correlated with ingestion of mercury,[190,200] especially through ingestion of mercury-contaminated fish, so diet plays an important role. Concentration variations have been noted among persons living under the same environmental conditions and among single hairs taken from the same individual. Differences in trace metals concentrations in hair have been noted with age, sex and hair color.[197] "Normal" mercury levels in hair can therefore depend on individual metabolism, geographic location, diet and personal hygiene habits among other things. Longitudinal concentration variations may be caused by specific exposure to

Table 22

Normal Mercury Concentrations in Scalp Hair

Location of Population	Analytical Method	(number of peopled sampled)	ppm Hg in hair mean	range	Reference
CANADA	NAA	776	1.8	0-19	53
NEW ZELAND	NAA	33	2.2	0.3-34	54
USA	NAA	33	1.8	0.5-5.3	54
JAPAN	Dithizone	94	4.2	0.9-12	55
NEW ZELAND	NAA	33	1.8	0.1-33	54
USA (males)	AAS	80	10.1		12
USA (females)	AAS	147	20.8		12
USA (males)	AAS	83	18.0		13
USA (females)	AAS	143	19.0		13
USA (males)	AAS	18	5.4		40
USA (females)	AAS	19	3.7		40
USA	AAS-quartz T	54	5.6	0.2-27.5	this study
USA (males)	AAS-quartz T	32	4.6	0.2-24.7	this study
USA (females)	AAS-quartz T	22	7.0	0.2-27.5	this study

a) NAA = Neutron Activation Analyses; AAS = Atomic Absorption Spectroscopy.

mercury, but may also be affected by, for example, physical abrasion of the hair matrix by brushing. Other factors which may affect the studies of "normal" mercury concentrations in hair include the sampling technique and the method of analysis.[195]

For example, a variation can be expected if hair is collected from clippings as opposed to plucking hair from specific regions of the head. Analysis of different parts of the hair shaft can cause significant variation.

It is also illustrated in Table 22 that neutron activation analysis gives lower data than methods involving atomic absorption or dithizone colorimetry. The former method involves a minimum of sample preparation, but significant post-excitation separation. The latter methods require sample pretreatment before analysis, such as oxidative digestion and preconcentration.

2. Problems in the Analysis of Hair

Analysis of hair presented many of the problems common to analysis of other biological tissues. These included low concentrations of the element of interest, limited quantity of sample available, and a complex, variable matrix. These analytical problems were usually overcome by destruction of the sample matrix followed by the determination of the element of interest. Destruction of the hair matrix was usually accomplished by dry-ashing in a muffle furnace or by wet digestion with concentrated mineral acid or strong oxidizing agents. Dry ashing was followed by dissolution of the residue to convert the element

of interest to a known chemical form. If necessary, concentration of the element from this solution was accomplished by ion exchange, chelation and extraction, or evaporation of the solvent. With volatile compounds like those of mercury, significant losses of the element can occur in these preliminary steps.[158] These losses may result in large negative errors in the determination. Large positive errors can also occur in these preliminary steps, through contamination of the sample by mercury which may be present in added reagents.

Errors in determined mercury concentrations can also result during storage of either the aqueous sample or the aqueous mercury standard solutions. Solutions of mercury salts have been shown to lose volatile mercury very readily.[30] Calibration curves prepared from deteriorated solutions will be inaccurate and result in falsely high sample concentrations. Similarily, stored samples may lose mercury, causing low answers to be obtained.

The hair sample can be contaminated by external sources such as sweat, applied hair preparations (shampoo) or dust. Normally, a washing procedure is employed prior to analysis. Hair is a natural ion-exchanger, however, and may lose ionic material for an indefinite number of washes,[201] so some authors advocated no washing at all.[202] Procedures for washing the hair sample prior to analysis are varied. Acetone, water, detergents, alcohols and ethers have been used, either alone or in combination. Recommended washing periods ranged from a few seconds to several hours.

3. Methods Used to Determine Hg in Hair

Many analytical methods have been used to determine mercury in hair. Methods in current use include colorimetry, neutron activation analysis (NAA) and atomic absorption spectroscopy (AAS). Colorimetric analysis is usually performed by measuring the absorption of orange mercury dithizonate in $CHCl_3$ at 490 nm. Hair samples are digested or ashed and the residue dissolved. Dithizone is added and the solution is made strongly acidic. Mercury dithizonate is extracted into $CHCl_3$ and the absorption by the $CHCl_3$ layer at 490 nm is measured. Dithizone is not a specific chelating agent for mercury, so interfering ions Cu,Ag, Au, Pd and Pt must be eliminated through the use of masking agents. The detection limit of this technique is reported to be approximately 5×10^{-8}g Hg.[152]

NAA has been used extensively in the determination of trace metals in hair.[198,202-205] The hair sample is sealed in a quartz or polyethylene vial and is irradiated. ^{197}Hg, a gamma emitter, is formed from mercury present in the sample and counted. The intact hair sample can be counted. The detection limit for non-destructive NAA is about 1.7×10^{-8}g Hg.[55] The detection limit can be lowered if post-irradiation dissolution of the sample and separation of mercury is performed prior to measurement of the gamma radiation emitted by ^{197}Hg. The detection limit is lowered[206] to approximately 2×10^{-10}g. This has become

a popular procedure. The enhanced sensitivity of destructive NAA has enabled workers to determine mercury concentrations in hair segments 5-cm long. Post-irradiation separation techniques include distillation of mercury and trapping the vapor on gold foil,[207] ion exchange,[208] isotope exchange,[209] and precipitation as the sulfide.[210] NAA has the great advantage of being a multielement technique, but some problems have been encountered in mercury determinations. Problems include loss of mercury during irradiation, interference from other radioactivities produced by matrix components and poor reproducibility.[211,212] A nuclear reactor is required for irradiation of the sample. Approximately 6 days are required for activation and counting of mercury in hair samples.

Inorganic and organic mercury compounds in hair have been determined by gas chromatography.[121] The organic mercury compounds are extracted into benzene from an alkaline digest of hair. The unextracted inorganic mercury is methylated with tetramethyl tin and then extracted into benzene. Bromide derivatives of the extracted compounds are formed, separated by gas chromatography and detected with an electron capture detector. The detection limit is about 1 ppb with 70-90% recovery of added mercury.

4. The Use of Atomic Absorption to Determine Mercury

AAS has been used widely in hair analysis. Both electrothermal and cold-vapor techniques have been applied

successfully to the determination of mercury in hair. Electrothermal atomization in a commercial graphite furnace has been used to measure directly trace metal concentrations in 1-cm hair segments, but mercury was not among the metals determined. Conventional electrothermal AAS analysis requires sample dissolution and preconcentration of mercury.

Analysis by the cold-vapor technique[53] is usually preceded by oxidation with permanganate. The mercuric ion remaining in solution is reduced to elemental mercury vapor by acidic stannous chloride. The released mercury vapor is swept into an absorption cell in the light path of an atomic absorption spectrometer. The absorption of the 253.7 mm mercury resonance line is measured.

Oxygen-filled combustion tubes have also been used to destroy the hair matrix. The released mercury vapor is trapped by amalgamation with gold foil.[214] Mercury is released from the gold foil by heating or electrolysis and the released vapor is swept into an absorption cell as above.

The detection limit of the cold vapor-AAS technique is about 6×10^{-10} g Hg. The technique has the advantage of being quick and inexpensive, but significant loss of volatile mercury and significant sample contamination can occur.

All reported AAS analyses of mercury in hair have used the spin-forbidden resonance line at 253.7 nm. It has been known that the 184.9 nm resonance line has an oscillator strength which is

forty-five times greater than the oscillator strength of the 184.9 nm line. Although the greater oscillator strength of the 184.9 nm line should lead to increased AAS sensitivity,[63] the line has not been used extensively in mercury determinations. Difficulty was encountered in working at 184.9 nm, in the vacuum-ultraviolet region, because of molecular absorption by both atmospheric oxygen and molecules like H_2 and CO which are produced during sample atomization. Successful exploitation of this line has been reported in the direct determination of mercury in air[64] and in the cold vapor technique determination of mercury in water and biological tissues.[107]

Because of the limitation inherent in any technique which requires preliminary destruction of the sample matrix or trapping and concentration of the element to be determined, a method for the analysis of mercury in hair was desired that would permit sensitive, accurate, reproducible determinations without any sample pretreatment. Such a method for direct analysis of biological tissues was readily available through the use of the quartz "T" atomizer for electrothermal atomic absorption spectroscopy. The quartz "T" atomizer has a direct analysis capability for solid hair samples and has been used for the analysis of cadmium in hair.[216] It allowed the use of the more sensitive vacuum-ultraviolet resonance line by the simple expedient of flushing the optical light path with nitrogen or some other non-absorbing gas. It was the purpose of this study to exploit

both of these advantages to enable the direct determination of mercury in the scalp hair of a non-occupationally exposed population.

B. EXPERIMENTAL

1. Equipment

The quartz "T" atomic absorption system has been described in Chapter 1.

2. Analytical Procedure

a. Operating Parameters

All hair analyses were performed at 184.9 nm. The operating parameters used were the same as those listed in Chapter 1 for work at 184.9 nm.

b. Sample Collection

Hair from males and females in the Louisiana State University population was analyzed. Chemistry faculty and graduate students constituted the bulk of the sampled group, but undergraduate students, secretarial personnel and children were included. None of the sampled population was occupationally exposed to mercury (other than normal laboratory exposure). Two of the subjects were currently working with elemental mercury on a limited basis. Six of the fifty-four subjects were daily cigarette smokers. Most subjects consumed fish two to four times per month.

Hair samples were obtained by plucking single hairs directly from the scalp. The hairs were placed between sheets of glassine

powder paper (Eli Lilly and Co.) until analysis. Hair samples were normally obtained on the day of analysis. Samples collected outside the laboratory were transported in plain white envelopes. Only those hairs which had a visible root terminus were analyzed. All sample-handling equipment was cleaned with 10% (v/v) HNO_3.

Single hairs were rinsed in a stream of deionized distilled water from a wash bottle to remove gross surface contamination. Some samples were analyzed without washing and some were rinsed in acetone to allow a comparison of washing procedures. Cleaned hairs were handled with tweezers.

Single whole hairs were cut into 1-cm segments using a razor blade. The root terminus and adjacent 0.5 cm of hair were cut off and discarded to avoid erroneous analysis of follicle material. The segments were weighed to the nearest 0.01 mg on a Mettler H-10 analytical balance. Segments generally weighed between 0.05 and 0.1 mg/cm. The precision of the weighing step was therefore between 10 and 20%.

A 1-cm segment was used because it produced an atomic absorption signal of approximately 10% and a molecular absorption signal of only about 5%. It was not desirable to increase the amount of hair taken for analysis. As can be seen from Figure 12, the calibration curve is not linear. It was desirable to keep the atomic absorption signal from the sample in the most linear region of the curve, between 15 and 30% absorption. Longer hair sections decreased the ability to observe longitudinal variations in

concentration. Longer hair segments also had a tendency to hit the heated wall of the inner sleeve above the carbon bed. They adhered to the wall and pyrolyzed there instead of on the carbon bed. This resulted in incomplete sample degradation.

c. Determination of Mercury

Analysis was performed by inserting a cleaned hair segment into the inner sleeve of the atomizer and dropping the segment directly onto the hot carbon bed. Care was taken to insure that the segment was inserted below the level of the purge gas inlet. Resonance absorption was measured at 184.9 nm with the demountable mercury hollow cathode lamp as the source. Background molecular absorption was measured at the same wavelength setting with the deuterium lamp as the light source.

Mercury concentrations were determined along the hair strand in 1-cm segments from the proximal (root) end to the distal end. The first and second segments from the root and the first and second segments from the distal end were averaged to give proximal and distal concentrations, respectively.

d. Calibration.

Calibration was performed using elemental mercury vapor. Air was saturated with distilled elemental mercury at room temperature in a series of septum-capped glass bottles. A 1-ml gas-tight syringe (Hamilton No. 1001) was used to inject various quantities of Hg-saturated air into the atomizer. Care was taken insure that the syringe tip was well below the purge gas inlet.

The weight of mercury in the saturated air was calculated from vapor pressure data.[127] For example, at 20.0°C, the vapor pressure mercury is 159.99 x 10^{-3} Pa, which is equivalent to 13.17 mg Hg/cm^3. The results of a series of these calculations are presented graphically in Figure 11. A typical calibration curve is shown in Figure 12. The sensitivity, defined as that quantity of mercury equal to 1% absorption, was 1.5 x 10^{-11}g and the relative standard deviation was $\pm$ 7%. Typical absorption signals for hair segments and standards are shown in Figure 37.

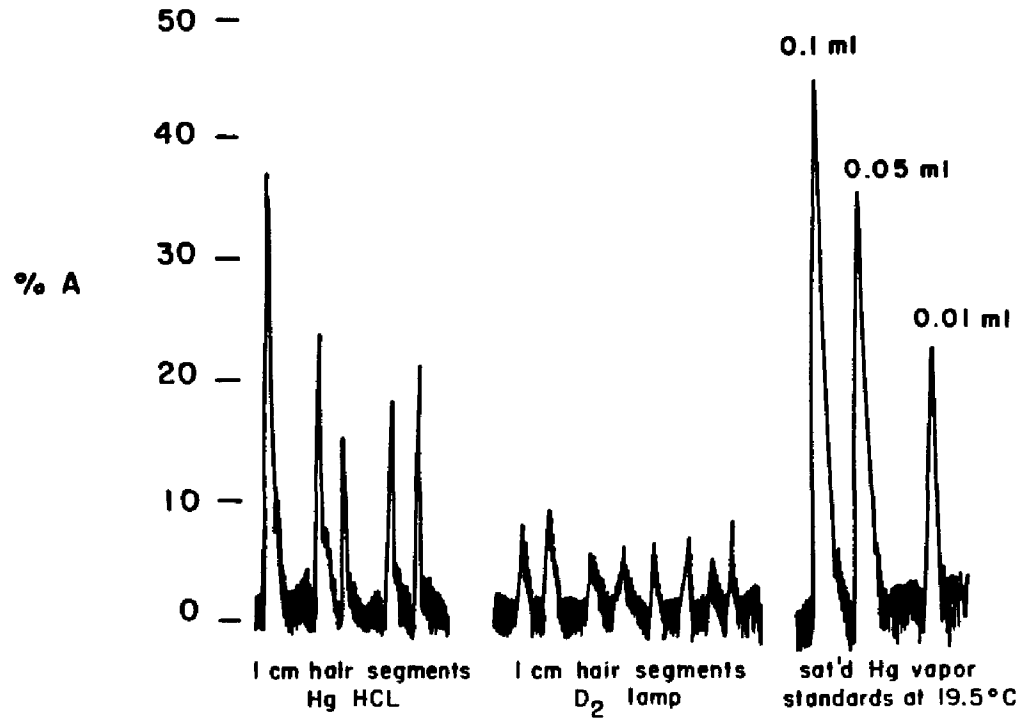
C. RESULTS

1. Variation in Mercury Concentration Along the Hair Shaft

Table 23 presents the average proximal and distal mercury concentrations found in 34 of the adults sampled. The average proximal concentration in females was 2.7 ppm Hg and in males, 0.9 ppm Hg. The average distal concentration in females was 5.9 ppm Hg and in males, 2.0 ppm Hg. Distal concentrations were about three times higher than proximal concentrations. Twenty-five percent of the sampled population, including all of the children sampled, showed either unchanged or decreased mercury concentrations with respect to distance from the scalp.

Two subjects who were working with elemental mercury showed mercury concentration spikes along the hair shaft.

FIGURE 37: RESONANCE (Hg) AND MOLECULAR BACKGROUND (D_2) ABSORPTION SIGNALS ARE SHOWN FOR 1-cm HAIR SEGMENTS DROPPED DIRECTLY ONTO THE HOT CARBON BED.



Typical signals from 1 cm hair segments and mercury standards at 184.9 nm

Table 23

Mercury Concentrations in Hair: Proximal Versus Distal

Female					Male				
Subject	Strand Length (cm)	ppm Hg Proximal	ppm Hg Distal	[Hg] Distal / [Hg] Proximal	Subject	Strand Length (cm)	ppm Hg Proximal	ppm Hg Distal	[Hg] Distal / [Hg] Proximal
1	30	3.2	9.6	3.0	1	5	0.1	0.5	5.0
2	10	0.6	1.1	1.8	2	10	0.5	0.3	0.6
3	5	1.2	6.5	5.4	3	8	0.1	2.5	19.0
4	20	0.8	4.2	5.0	4	13	0.4	0.4	1.0
5	19	1.0	2.8	2.8	5	10	0.9	0.9	1.0
6	8	2.1	13.1	6.1	6	11	1.2	0.7	0.6
7	20	7.5	15.3	2.0	7	6	0.6	2.0	3.3
8	20	8.3	5.8	0.7	8	7	0.7	1.5	2.2
9	20	0.7	1.7	2.5	9	12	1.5	4.0	2.7
10	19	0.8	3.8	4.6	10	6	1.5	2.9	1.8
11	19	4.3	2.8	0.7	11	10	0.3	1.5	5.4
12	30	2.8	11.7	4.1	12	10	1.2	6.5	5.4
13	30	1.0	2.3	2.3	13	8	2.1	6.7	3.1
14	28	1.3	1.7	1.2	14	5	1.6	0.5	0.3
15	29	3.3	10.0	0.8	15	10	0.8	1.2	1.5
16	28	3.2	2.7	0.8	16	11	1.9	0.7	0.4
17	27	3.6	5.2	1.4	17	5	0.7	1.7	2.5
Female avg. ($\bar{x}$):	21	2.7	5.9	2.8	Male avg. ($\bar{x}$):	9	0.9	2.0	3.3
σ:		2.3	4.4	1.7	σ:		0.6	2.0	4.4
Overall average (x):	15						1.8	4.0	3.0
σ:							1.5	3.2	3.3

2. Variation in Mercury Concentration Among Hairs from An Individual

Twenty hairs from one individual were analyzed on the same day. The average distal and proximal mercury concentrations were calculated, as was the standard deviation in the results. The results are presented in Table 24. The average proximal concentration was 3.4 ppm Hg with a standard deviation of 1.0 ppm and a range of 2.0-5.5 ppm. The average distal concentration was 5.4 ppm, with a standard deviation of 2.5 ppm and a range of 2.9-13.3 ppm.

3. Range of Mercury Concentration In the Hair of Non-Occupationally Exposed Individuals

Table 25 presents the average mercury concentrations found in scalp hair taken at random from non-occupationally exposed subjects. The average mercury concentration for females was 7.0 ppm Hg and for males, 4.6 ppm Hg. The overall average mercury concentration was 5.6 ppm, with a range of 0.2-27.5 ppm.

4. Variation in Mercury Concentration in Hair with Age

Mercury concentrations in hair with respect to the age of the subject sampled are presented in Table 26.

5. Variation in Mercury Concentration in Hair with Hair Color

Mercury concentrations in hair with respect to hair color of the subject sampled are presented in Table 27.

6. The Effect of Washing Procedures on Mercury Concentrations in Hair

The effect of washing hair with deionized distilled water

Table 24

Distal and Proximal Mercury Concentrations in Different Hair Strands for One Individual

Proximal: Hg (ppm)	Distal: Hg (ppm)
5.3	6.0
2.9	4.7
4.0	4.7
4.0	3.1
2.9	4.7
2.7	8.0
2.4	13.3
2.7	5.3
2.7	8.4
2.7	4.0
2.0	3.6
3.3	3.9
2.7	2.9
3.6	5.2
5.5	3.6
3.6	2.9
3.6	5.2
2.0	8.0
4.7	5.2
4.7	5.3
number of hairs sampled) = 20	20
x (average) = 3.4	5.4
standard deviation = 1.0	2.5
range = 2.0-5.5	2.9-13.3

Table 25

Average Hg Concentration in Randomly-Sampled Hair from a "Normal" Population

		ppm Hg		
		Male	Female	Overall
number of people sampled:	n	32	27	54
average concentration:	$\bar{x}$	4.6	7.0	5.6
standard deviation:	σ	6.3	8.2	7.1
	range	0.2-24.7	0.2-27.5	0.2-27.5

Table 26

Variation in Average Hg Concentration with Age

Age (years)	(number of people sampled)	avg. Hg conc. (ppm)
0-9	6	<0.4
10-19	10	2.3
20-29	22	7.4
30-39	5	10.4
40-49	6	3.6
50-59	5	7.5

Table 27

Variation in Average Hg Concentration with Hair Color
(Subjects >20 yrs. old)

color	(number of people sampled)	avg. Hg conc. (ppm)
Brown	24	9.5
Blond	3	1.8
Black	6	1.8
Red	1	6.1
Gray	4	6.2

Table 28

Effect of Washing Procedures on Proximal Mercury Levels

Sample	Treatment	ng Hg/cm hair found
I	unwashed	0.1
	acetone wash	0.2
	deionized distilled water wash	0.1
II	unwashed	0.4
	acetone wash	0.5
	deionized distilled water wash	0.5
III	unwashed	0.4
	acetone washed	0.3
	deionized distilled water wash	0.3

or acetone on mercury concentrations was investigated. The results are presented in Table 28.

7. The Concentration of Mercury in Commercial Shampoo

Five commercial shampoos were analyzed for mercury using the carbon disk technique described in Chapter 1. One shampoo was found to contain 90 ppm Hg. No mercury was detected in the other 4 shampoos (<0.05 ppm Hg). Atomic absorption was measured at 253.7 nm because of high background absorption (>80%) at 184.9 nm.

D. DISCUSSION

1. Advantages of the Method

The use of the quartz "T" atomizer in the determination of mercury in hair has several advantages over other methods of analysis.

a. It permitted exploitation of the more sensitive 184.9 resonance line. This resulted in a sensitivity which is at least one order of magnitude greater than competing techniques.

b. It eliminated the need for sample pretreatment, due to the ability of the atomizer to efficiently decompose solid samples. The time required for the determinations was therefore greatly reduced.

c. Accuracy was improved because there was no loss of mercury due to volatilization during dry ashing procedures, incomplete digestion or incomplete recovery from concentration steps. In addition, no mercury contamination occurred from

reagents used for wet ashing.

That complete degradation of the hair matrix occurred under prevailing analytical conditions was shown during a previous study on cadmium levels in hair.[216] No molecular absorption was seen at 226.6 nm, an off-resonance line for cadmium, for 1-cm hair segments, which indicated complete breakdown of the matrix. This was confirmed during this study by observing that no molecular absorption occurred at the 253.7 nm mercury line. Although molecular absorption was observed at 184.9 nm, it was due to the formation of combustion products such as H_2 and CO which absorb in the vacuum-ultraviolet region.

d. The positive pressure throughout the atomizer insured that no mercury present in a sample could exit from the system without passing through the light path. Absorption of mercury on the quartz walls of the heated atomizer is considered to be highly unlikely. Coupled with the direct solid analysis capability, these advantages are considered to increase greatly the accuracy of the determinations.

e. Complete decomposition of the sample matrix allowed the use of mercury vapor-saturated air for calibration. The calibration procedure was simple, convenient and eliminated errors due to degradation of stored aqueous standard solutions.

f. The sensitivity of the quartz "T" atomizer permitted the analysis of very small (1-cm or less) segments of hair. The longitudinal variation of mercury concentration in a

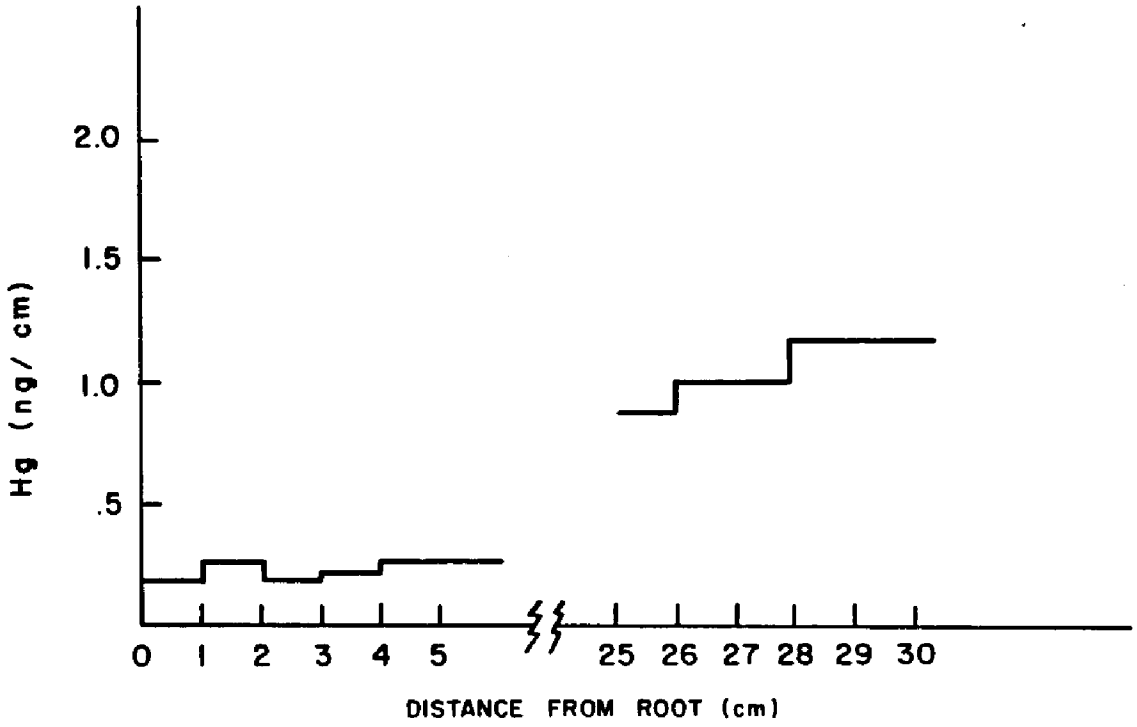
single hair was able to be studied more thoroughly than had been done previously.

2. Variation in Mercury Concentration Along the Hair Shaft

The very small segments of hair analyzed in this study allowed ready observation of previously reported longitudinal variations in mercury concentrations. A general, but not universal, trend to higher mercury concentrations in the distal end than in the proximal (root) end was noted. Seventy-five percent of the individuals sampled showed higher distal than proximal mercury concentrations. The twenty-five percent who showed either no change or a decrease in mercury concentration with distance from the scalp included all of the children sampled. Distal concentrations were about three times higher than proximal concentrations. This trend is illustrated in Figure 38, a composite of mercury concentrations in 10 single hairs from one subject. The unit of the ordinate in Figure 38 is absolute weight of mercury, which demonstrated that the increased distal concentration was not an artifact due to thinning of the hair shaft with distance from the scalp.

The two subjects who were using elemental mercury in their research showed mercury concentration peaks along the hair shaft in contrast to the steady increase in mercury concentration seen in other subjects. This can be seen in Figure 39, which presents concentration versus length for the two subjects working with mercury and for two subjects, of similar age, hair length and sex

FIGURE 38



MERCURY CONCENTRATION IN HAIR: LONGITUDINAL VARIATION
COMPOSITE OF 10 HAIRS FROM SAME INDIVIDUAL

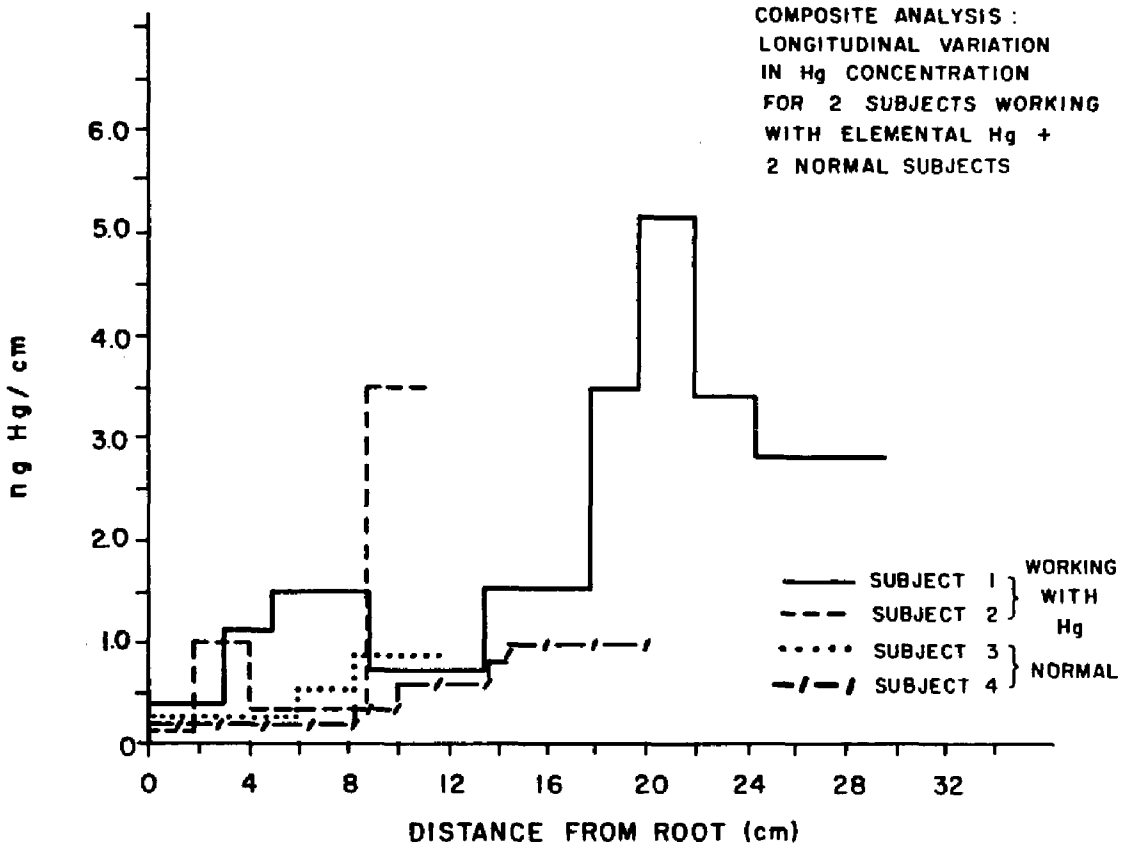


FIGURE 39: IN SUBJECTS 1 and 2, WHO WORKED WITH MERCURY, LARGE JUMPS IN MERCURY CONCENTRATIONS ALONG THE HAIR SHAFTS WERE SEEN (IN cm 8-12 FOR SUBJECT 2; IN cm 4-8 AND 18-22 FOR SUBJECT 1). NORMAL SUBJECTS 3 AND 4 SHOWED ONLY A GRADUAL INCREASE IN MERCURY CONCENTRATIONS ALONG THE HAIR SHAFT.

to the first two, who were not working with mercury. The concentrations in the hair of the subjects working with mercury were the highest concentrations found in the 54 subjects analyzed. These results indicated that mercury levels in hair serve as a chronological record of exposure.

Because of obvious environmental influence on the concentration of mercury in hair, only the mercury concentration in the segment nearest to the root would be most representative of mercury actually excreted from the body.

The average proximal mercury concentration found in females was 2.7 ppm and in males, 0.9 ppm. This difference would seem to indicate a difference between the sexes in the excretion of mercury in hair. A more likely explanation of the higher mercury levels in female hair is a difference in exposure to mercury through the usually different hair grooming products used by each sex.

The overall average proximal mercury concentration found in this study was 1.8 ppm or 1.3×10^{-10} g/cm. It is possible to estimate the amount of mercury excreted by the body through scalp hair by using this proximal concentration. It has been estimated that a normal human will grow approximately 40 m of scalp hair in one day.[217] Therefore, approximately 0.5 μg of mercury/day will be excreted by the body in scalp hair.

3. Variation in Mercury Concentrations Among Hairs from An Individual

It was observed that the measured concentration of mercury varied slightly for different hairs from the same individual. Hairs were plucked from the crown of the head in order to minimize composition differences which might have arisen due to location of the hair. Twenty hairs of approximately the same length (27-30 cm) were chosen. The twenty hairs were analyzed on the same day. The average distal and proximal concentrations were calculated as described previously. The results were given in Table 24. The average proximal concentration was 3.4 ppm, with a standard deviation of 1.0 ppm, and a range of 20-5.5 ppm. The average distal concentration was 5.4 ppm, with a standard deviation of 2.5 ppm and a range of 2.9-13.3 ppm.

The standard deviation of the results is a measure of both the indeterminate error and variations in composition between different segments. The variation seen was not due only to random error since each segment analyzed was unique. Therefore no measure of the short term precision of the procedure is available, but the relative standard deviaion is expected to be no better than that of the vapor standard, which is 7%.

It is believed that most of the variation observed between single hairs of an individual was due to differences between the hairs themselves. Mercury concentration differences could arise from differences in the thickness and texture of the hair segment,

for example. Hair tended to contain more natural oil near the root than at the distal end. Physical damage to the hair shaft through brushing, blow-drying and so on was more pronounced at the distal end than at the proximal end. In many cases, individual hairs from the same subject were different colors. Variations may occur in the exact composition of the protein matrix in a given strand; for example, curly and straight hairs may be found on an individual. All of these factors can be contribute to differences in mercury concentrations by affecting bonding sites in the hair shaft.

4. Range of Mercury Concentrations in the Hair of Non-Occupationally Exposed Individuals

Most determinations of trace element levels in human hair have been made on randomly collected, batched and homogenized samples. Random hair segments also more closely represent real samples presented for analysis in a clinical or forensic laboratory. Therefore, in order to compare results obtained with the quartz "T" atomizer with previously reported methods, 54 hair samples were analyzed by taking four 1 cm segments at random from a single hair. The mercury concentration in each segment was determined and the results were averaged. These results are presented in Table 25 and Figure 40.

The average mercury concentration for the entire group of males and females was 5.6 ppm. The concentrations found ranged from 0.2-2.75 ppm.

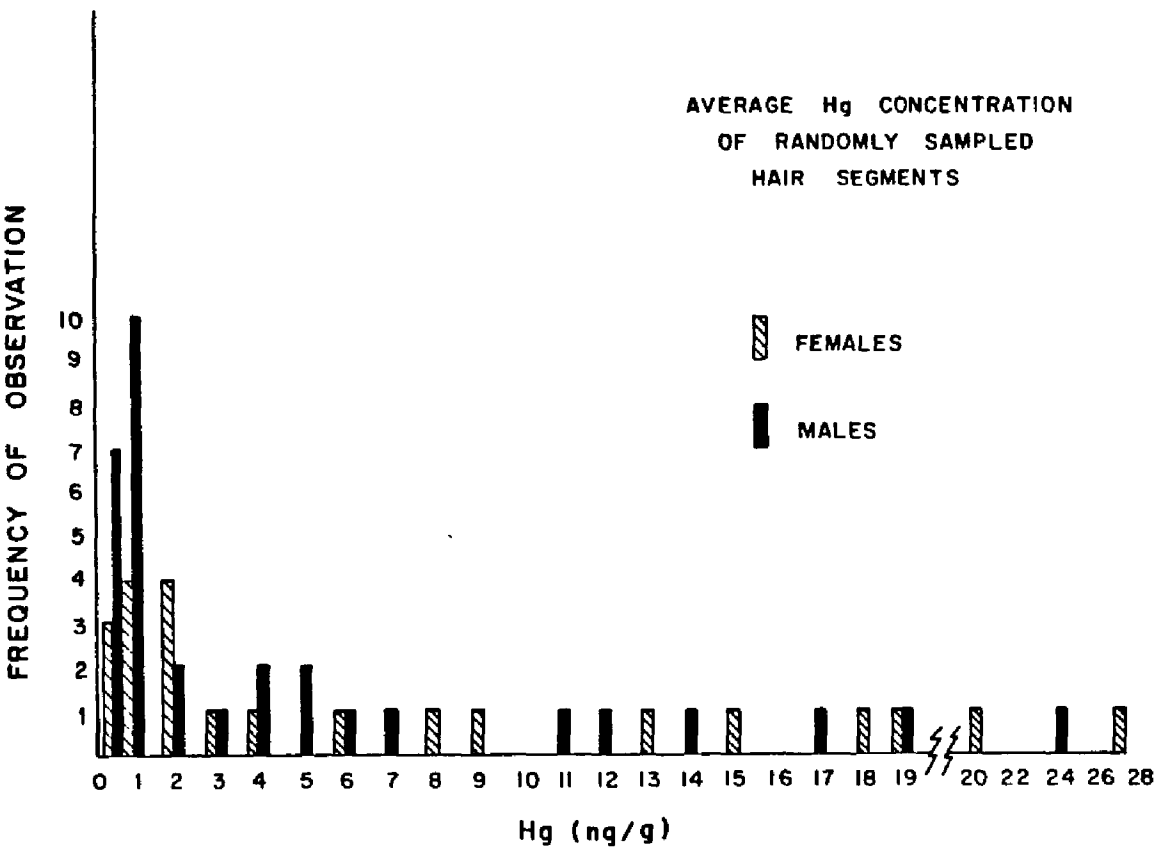


FIGURE 40: DISTRIBUTION OF MERCURY CONCENTRATIONS IN HAIR.

5. Variation in Mercury Concentrations with Age

Mercury concentrations in hair appeared to vary with age among the population sample, although such a conclusion cannot be drawn with certainty from the small sample of people taken.

The mercury in the hair of the six children who were less than 10 years old was equal to or less than the detection limit of 1×10^{-11} g. From this, it was calculated that the average concentration in the hair of the six children was ≤ 0.2 ppm.

Mercury concentrations increased with increasing age from 10 years to 39 years of age, then appeared to decrease slightly. A smooth curve fitted to the data showed that mercury concentrations leveled off at approximately 7.5 ppm. This is believed to represent attainment of a steady state between exposure and excretion. The data are presented in Table 26.

6. Variation in Mercury Concentration with Hair Color

As can be seen in Table 27, most of the adults sampled had brown hair. Brown hair appeared to have highest mercury concentrations, 9.5 ppm. Gray and red hair contained about 6 ppm mercury, while blond or black hair contained only 1.8 ppm mercury. None of the subjects had used hair dyes or coloring preparations.

7. The Effect of Washing Procedures on Mercury Concentration in Hair

Various washing procedures have been recommended in the literature for removal of surface contamination from hair. Previous studies using both organic solvents and deionized water

to wash hair samples found no change in the mercury concentrations in hair after washing.[198,204,205]

In this study, several hairs from three subjects were rinsed in a stream of deionized distilled water in the usual manner. A second sample was rinsed in a stream of acetone. A third parallel sample was not washed. Hairs were cut and weighed as usual. No difference in segment weight as a result of washing could be detected among the three samples from the same subject. Only the first three segments at the proximal end were analyzed.

No significant difference in proximal mercury concentration was found between the washed and unwashed samples. The data are presented in Table 28.

Longitudinal concentration profiles of unwashed, acetone-washed and distilled deionized water-washed hairs from the female subject who was working with elemental mercury were determined. Figure 41 shows that the proximal mercury concentrations were not affected by washing. The distal concentrations appeared to be lowered by washing with water and with acetone.

8. The Concentration of Mercury in Commerical Shampoos

Five representative commerical shampoos, including a children's shampoo and a dandruff shampoo, were analyzed for mercury content to ascertain whether exposure differences could occur through personal grooming habits.

One shampoo contained 90 ng Hg/μL. No mercury was detected in four shampoos <0.01 ng Hg/μL). It appeared that choice of

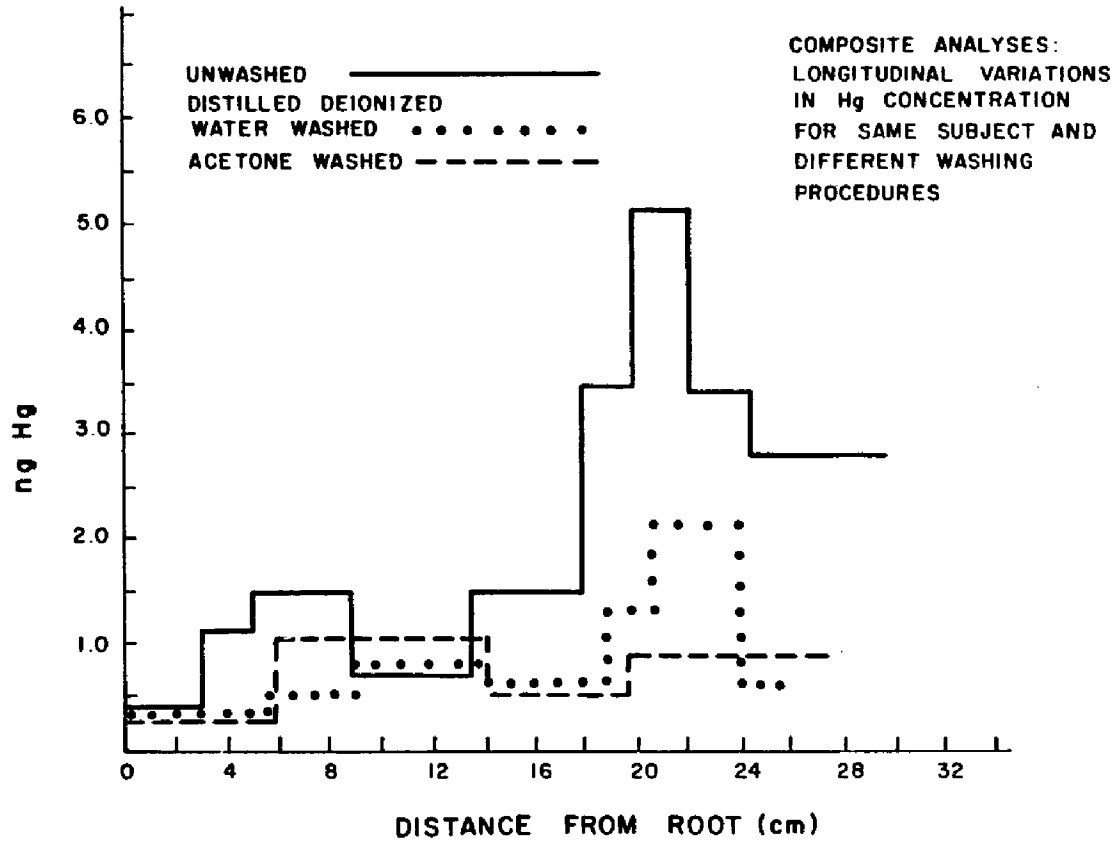


FIGURE 41: THE EFFECT OF WASHING PROCEDURES ON MERCURY IN HAIR. DEIONIZED WATER AND ACETONE WERE EACH EFFECTIVE IN REMOVING MERCURY FROM HAIR. WASHING WAS MORE EFFECTIVE IN REMOVING MERCURY AT THE DISTAL END OF THE HAIR (PRESUMED TO BE MAINLY EXTERNALLY-ABSORBED MERCURY) THAN IN REMOVING MERCURY AT THE PROXIMAL END (PRESUMED TO BE INTERNALLY-EXCRETED MERCURY).

shampoo could significantly increase the exposure of scalp hair to mercury.

9. Correlation Between Mercury in Urine and in Scalp Hair

Nine subjects had mercury levels in both urine and scalp hair determined in this study. It was of interest to see if a correlation existed between the two mercury levels. Urine mercury concentrations were plotted against the hair root mercury concentrations. The correlation coefficient was calculated to be 0.158. It was not very surprising that no correlation existed between these two measurements, for the following reason. One centimeter of hair represented about 25 days' growth and would be a time-weighted average mercury level, whereas a urine specimen was a one-day random sample. As was discussed in Chapter 2, the urine mercury concentration of an individual fluctuated greately on a day-to-day basis. A more informative correlation could be calculated from an average mercury concentration in urine taken over a one month period. Such a study was made for only two subjects, which was an insufficient number for a meaningful calculation.

E. CONCLUSION AND SUMMARY

The use of the quartz "T" atomizer for atomic absorption spectroscopy enabled the development of a simple, sensitive, effective method for the direct determination of mercury in hair. The use of the 184.9 nm line permitted increased sensitivity over the more commonly employed 253.7 nm line. Complete decomposition

of the sample allowed the use of elemental mercury vapor for calibration purposes, which eliminated problems with unstable aqueous mercury solutions.

The average mercury concentration in hair of a non-occupationally exposed population was determined to be 5.6 ppm.

Mercury concentrations in hair generally increased with increasing distance from the root. Therefore, mercury levels in hair segments nearest the root most closely approximated the amount of mercury excreted from the body. The mercury concentration at the hair root could be used to indicate the body burden of mercury in an individual provided that individual variations are taken into account.

CHAPTER 6

THE DIRECT DETERMINATION OF MERCURY IN BREATH AND SALIVA

A. INTRODUCTION

1. The Use of Breath and Saliva as Biological Indicators of Exposure to Mercury

Symptoms of both acute and chronic mercury intoxication include a variety of oral and respiratory problems. Examples are salivary gland swelling, excessive salivation, metallic taste, foul breath, loose teeth, soft, spongy gums, a blue-black line around the gums due to a mercury-sulfhydryl complex and rapid respiration.[2,218-220] Consequently, breath and saliva appear to be likely biological indicators of exposure to mercury.

a. Breath

A breath sample possesses many advantages for clinical analysis or occupational health studies as discussed at length in a review by Dubowski.[221]

Breath is a convenient sample to monitor since it can be collected rapidly with no inconvenience to the subject. Gaseous samples require little or no pretreatment. Portable detectors are available for many gaseous substances, so breath sampling can provide a real-time "field" measurement. Breath samples can also be trapped on an adsorbant for later analysis.

Breath samples reflect the concentration of substances transferred across the alveolar-capillary membranes. A continuous

equilibrium is established for gases and volatile substances between the alveolar air and pulmonary circulation. Therefore, breath analysis reflects the blood level of substances such as CO_2, alcohol, CO, anesthetic gases, sulfides and volatile metals.

It is well-known that elements like Se and Te can be volatilized from the lungs as methylated compounds following oral ingestion of inorganic Se and Te compounds. It is also well-known that inorganic mercury can be methylated by bacteria. It is feasible that mercury or methylated mercury could be excreted from the body in the breath.

Breath has been studied by a number of researchers, to determine its suitability as an indicator of exposure to mercury and to elucidate the metabolism of mercury in the body.

Several animal studies indicated that rats can volatilize (from the lungs and body surface) mercury intra-cardially or intravenously injected.

Clarkson and Rothstein[222] found that a volatile mercury compound was exhaled by rats after injection with radioactive $^{203}Hg(NO_3)_2$. In the first hours after injection, when mercury levels in blood were high, excretion from the lungs was high. Thereafter, excretion was about equally divided between lungs and body surface. Ten percent of the total mercury excreted on the first day was through the lungs and body surface. Lungs and body surface accounted for about 4% of the total amount

excreted over the course of the experiment.

Magos[223] found mercury in the breath of rats injected with either radioactive elemental mercury or mercuric ion, but the amount exhaled varied with the chemical form of the mercury injected. About 20% of the Hg° dose was exhaled within 30 seconds of injection. Only 1.8% of the Hg^{2+} dose was exhaled.

In both of the above studies, the chemical form of the exhaled mercury was not determined. Breath samples were trapped on an adsorbant and the radioactivity was measured. Ostlund[80] investigated the metabolism of dimethylmercury in mice after inhalation or intravenous exposure. The major part of the dose was rapidly exhaled as dimethylmercury, with 80-90% of the dose eliminated in 6 hours. The identification of the chemical form of exhaled mercury was done by thin layer chromatography (TLC).

No evidence of the volatilization of inorganic mercury through the lungs has been found for humans. Studies of mercury levels in human breath are limited and most involve exposure of humans to elemental mercury vapor.

In 1941, Shepard et al.[224] reported the almost quantitative removal of Hg vapor from respired air by measuring ambient air Hg° concentrations and Hg° in exhaled breath. This can be explained in terms of the lipid solubility and high diffusibility of Hg°. It has been calculated that at 40°C the partition coefficient of mercury between air and body lipids is about 1:20, in favor of the body.[225] The results of Shepard

et al.[224] have been confirmed by other workers[226-228] who found that 75-85% of inspired Hg° (concentrations ranging from 50-350 $\mu g\ Hg/m^3$ air) was retained in the human body. These results were also obtained by measuring mercury vapor concentrations in ambient (inspired) air and exhaled breath. In these studies, elemental mercury vapor was inhaled and elemental mercury vapor was the chemical form detected in exhaled breath. Usually, a UV photometer set at 253.7 nm was used detect exhaled Hg°. It is postulated that inhaled Hg° rapidly crosses the alveolar membrane and, in a dissolved metallic form, diffuses into the red clood cells where it is oxidized into Hg^{2+}. Some dissolved elemental mercury persists in the blood long enough reach the brain.

Shoemaker[229] reported evidence that two humans receiving mercurial diuretics orally had mercury detected in their breath, but no details of the determination were provided. That the mercury detected came from diuretic retained in the mouth cannot be ruled out.

Hursh et al.[17] conducted an experiment in which 5 humans inhaled stable and radioactive mercury vapor for periods of about 20 minutes. Seventy-four percent of the inhaled dose was retained by the subjects, with retention occurring almost entirely in the alveoli. Breath samples were taken for 3 days following exposure. On the average, 7% of the mercury retained was exhaled in the breath, with a half-life of 18 hours. A plot of mercury exhaled versus time consisted of two components. The first component, including mercury flushed out of the lung dead space, was lost very

rapidly. The second component showed a more gradual loss of mercury. The authors postulated that mercury could be retained, and subsequently released in, the alveolar air space over a period of several days following exposure. This was thought to give rise to the second component.

Whole-body counting experiments gave an average half-time of 2 days for clearance of mercury from the lungs. Reinhardt et al.[230] measured mercury vapor in the breath of dental patients. Use of silver-mercury amalgam fillings in dental restoration is based on their inertness, permanence, abrasion resistance and fungicidal effect.[17] Large amounts of mercury vapor were exhaled by patients following the removal of old amalgam fillings by drilling. A plot of mercury exhaled versus time showed two components, in agreement with Hursh et al.[17] The fast loss component lasted for 30 minutes following removal of the fillings and amounted to about 2.4 μg Hg exhaled. The slow component, measured for the next six hours, amounted to about 5.3 ng Hg. The half-time of loss was about 4.2 hours. The authors estimated that 169 μg of Hg is retained by a patient upon the removal of an old filling.

It is evident that recent dental work can contribute significantly to mercury vapor levels in breath. Goldwater[219] stated that amalgam dental fillings do contribute to daily absorption of mercury by the body. No references could be found in which "normal" mercury levels in breath were measured.

Mercury in breath can reflect recent exposure, as shown in the above studies. Breath mercury levels should be considered in any discussion of elimination of mercury from the body. The suggestion has been made by Stopford[220] that an equilibrium exists between inhaled Hg° and Hg^{2+} in blood, so that Hg° is always available to cross the lung membrane and be exhaled.

b. Saliva

Saliva has not been extensively investigated as a biological indicator of exposure to mercury. The usefulness of saliva for this purpose is a matter of some debate. Studies performed in the 1920's found no significant amounts of mercury in saliva.[10,229] Berlin[18] stated that Hg^{2+} is accumulated in salivary and sweat glands, and that saliva, tears and sweat are routes of excretion for Hg^{2+}. Joselow et al.[231] found that saliva appeared to reflect the concentration of mercury in blood and therefore could be a physiological fluid of great diagnostic importance. They noted, however, that most saliva is reswallowed and the mercury reabsorbed, so that saliva is not an effective route of excretion for mercury. Stopford[220] stated that no mercury was found in saliva unless an exposure to mercury vapor had occurred, but that such mercury levels could be much higher than mercury levels in blood.

Excessive salivation and salivary gland enlargement are symptoms of mercury poisoning, but are more common on exposure to inorganic mercury than to organo-mercury compounds.[4] Work in

the 1920's on patients injected with mercury and mercury compounds (calomel, mercuric chloride) as treatment for syphilis found very small quantities of mercury in saliva.[10,229] In a study of industrial exposure, Joselow et al.[231] analyzed the saliva of exposed workers and normal adults. The concentration of mercury in the saliva of normal adults was <0.5 μg/100 mL. Concentrations in the saliva of exposed workers ranged from 1-15.5 μg Hg/100 mL with a mean of 5 μg Hg/100 mL. In the exposed group, saliva levels were about one-tenth of the urine concentrations. A better correlation was found between mercury levels in blood and in saliva mercury levels than between mercury levels in urine and in saliva mercury levels.

Stopford[220] reported finding an average level of 59 ppm Hg (range: 1-436 ppm) in saliva of workers at a chemical plant producing mercury compounds.

2. Difficulties in the Analysis of Breath and Saliva

a. General Considerations

Breath and saliva samples contain only trace (<ppm) amounts of mercury. Care must be taken to prevent contamination of the sample in the collection process. Loss of volatile mercury compounds must be prevented during storage and analysis of the sample. Preconcentration of the mercury in the sample is necessary for many analytical techniques and loss of mercury or contamination can occur during this process.

b. Breath

Only substances which pass through the circulatory-alveolar boundary can be determined in breath. The sample must be collected under conditions of known temperature, pressure and flow rate to accurately determine concentration of analyte and sample volume.

Breath has been shown to contain a wide variety of organic and inorganic gases.[221] Potential interferences from these molecules (e.g., molecular absorption of an atomic spectral line) must be considered in the analysis. There is a difference in chemical composition between lung dead-space air and alveolar air. This difference has been used to explain the inflection point in plots of mercury exhaled versus time from exposure.[17,230]

Collection and storage of breath samples[221] in foil containers, gas bags, glass gas pipets or adsorption onto activated charcoal, silica gel, etc., can lead to loss of mercury by adsorption onto container walls, by diffusion out of the container or by inefficient trapping.

c. Saliva

Saliva presents many of the same analytical problems as other biological fluids: limited sample availability, low concentration of analyte and a complex matrix. Sample digestion and concentration are usually required, with the concomitant risks of mercury loss or contamination.

Saliva is produced by the parotid, submaxillary and sub-

lingual glands. The chemical composition of saliva depends on the gland producing it an on the intensity, duration and type of stimulation.[232] Table 29, containing data reported by Afonsky,[232] presents some of the compounds and elements found in unstimulated saliva and their typical concentration ranges. Saliva also contains such things as epithelial cells, leucocytes, red blood cells and bacteria which add to the complexity of the matrix and change the chemical composition.

Various methods have been used to collect saliva for analysis.[233] Spitting, suction or simple drainage from an open mouth have been used to collect whole saliva. Special suction devices are used for collection of saliva from a particular gland.[233]

3. Common Analytical Methods for the Determination of Mercury in Breath and Saliva

a. Breath

Methods used for the determination of mercury in breath include radiotracer methods, UV-photometry and vapor phase atomic absorption spectroscopy.

Breath samples from subjects exposed to radioactive mercury were usually collected on Hopcalite (active MnO_2/CuO) or activated charcoal. The adsorbant was counted in a well-type scintillation counter.[17,222,223] Ostlund[80] used inorganic mercury and dithizone solutions to absorb exhaled radiolabelled organomercury compounds. The solutions were analyzed by low-

Table 29

Selected Components of Unstimulated Saliva[232]

Constitutent	Typical Concentration
Total Solids	240 - 1500 mg%
Organic Solids	130 - 380 mg%
Ash	55 - 370 mg%
Ca	2.5 - 11 mg%
P(total)	15 - 25 mg%
Na	1 - 65 mg%
K	30 - 95 mg%
Cl	30 - 145 mg%
Mg	0.1 - 0.7 mg%
citrate	0 - 2.0 mg%
thiocyanate	0 - 0.31 mg%
Fe	0 - 0.6 ppm
Cu	10 - 47.5 mg%
Co	0 - 12.5 ug%
F	0 .08 - 0.25 ppm
S	3 - 20 mg%
Br	0.2 - 7.1 ppm
total protein	140 - 640 mg%
cystine	0 - 45 mg%
glutamic acid	.2 - 12.5 mg%
methionine	0 - 0.1 mg%
glutathione	15.4 mg%
Vitamin B_{12}	0.15 - 5 ppb

temperature TLC and gamma counting. The detection limit was 2-80 pg Hg.

Nielsen Kudsk[227,228] used a commercial UV-photometer to measure Hg vapor in respired air. Two condensers at 11°C were used to remove water vapor from the breath before it passed into the photometer cell. A sensitivity of 3 μg Hg was reported.

Reinhardt et al.[230] passed breath samples through a drying tube and over a silver wool collector. Mercury was released from the silver wool by heating and the vapor was drawn through a quartz-cell atomic absorption system.

b. Saliva

Joselow et al.[231] determined mercury in saliva with a UV-photometer. Saliva samples were digested using a cold digestion and then extracted with dithizone. The solvent was evaporated and the residue heated in a furnace. The released mercury vapor was drawn through the photometer cell.

Stopford[220] gave no details of the analytical method used in his measurement of saliva mercury levels in industrially-exposed workers.

4. Speciation of Mercury Compounds in Breath and Saliva

Very little work has been done to identify the chemical form of mercury in breath. Most studies have measured the fractional amount of Hg vapor retained by the lungs as a function of mercury vapor in inspired air or have measured only the amount of expired radioactive mercury, not the chemical form. Only

Ostlund's TLC method[80] allowed identification of the chemical form of mercury exhaled by mice. By varying the absorbing solutions and TLC conditions, he was able to separate a number of inorganic, alkyl and aryl mercury compounds. Ostlund found no exhaled radioactive mercury from mice injected with ^{203}Hg-labelled methyl mercury hydroxide. After injection with ^{203}Hg labelled dimethylmercury, most of the dose was exhaled within one hour of injection as intact dimethylmercury.

The chemical form of mercury exhaled in human breath is not known. It could be in the form of dimethylmercury, analogous to the excretion of Se and Te in breath. Clarkson and Rothstein[222] suggested that it is probably in the elemental state since reducing agents present in tissue can reduce Hg^{2+} to Hg°. It is possible that some elemental mercury in breath arises from silver amalgam dental fillings. Microorganisms in the mouth could volatilize mercury. Stopford[220] suggested that some Hg° is always available to be exhaled from an equilibrium between Hg^{2+} and Hg° in the blood.

Speciation of mercury compounds in air was reported by Johnson and Braman.[234] They used a series of selective adsorption tubes to separate mercuric compounds, methylmercuric compounds, elemental mercury vapor and dimethylmercury. The sections of the adsorption train were heated to remove the various mercury species and the vapor passed into a helium dc discharge. Mercury was detected by measuring emission at the 253.65 nm line.

A detection limit of 5×10^{-12} g Hg was reported. It seemed feasible to us to apply this selective adsorption technique to the speciation of mercury in breath. Our attempt is discussed below.

No reports of speciation of mercury compounds in saliva were found.

5. Goals of This Study

The goals of this study were to develop a direct method for the determination of mercury in breath and saliva and to develop a method for speciation of mercury compounds in breath and saliva.

Atomic absorption spectroscopy using the Robinson quartz "T" atomizer fulfilled the requirements for mercury determinations in breath and saliva. The advantages of the quartz "T" atomizer for this type of analysis were numerous:

a. The long light path of the atomizer increased the sensitivity of the technique over conventional atomizers and made preconcentration of samples unnecessary.

b. The atomizer can be flushed with Ar or N_2, permitting use of the more sensitive spin-allowed mercury resonance line at 184.9 nm.

c. The sample was in contact with the carbon bed under a reducing atmosphere for about 30 seconds. This resulted in more efficient atomization of complex biological matrices.

d. The carbon bed can be composed of activated carbon for direct trapping of samples in the atomizer.

B. EXPERIMENTAL

1. Equipment

The atomic absorption system used for these studies was described in Chapter 1. Minor modifications were made as follows:

For breath sampling, the carbon bed was composed of activated National carbon[235] in order to adsorb mercury compounds from breath.

In addition, the top of the quartz "T" atomizer was fitted with a one-holed rubber stopper. A short piece of glass tubing was inserted through the stopper and a 30-cm length of Tygon tubing was attached to the glass. Subjects exhaled through the Tygon tubing directly onto the atomizer carbon bed.

For liquid sample introduction with the Drummond microdispenser, the top of the atomizer was fitted with a one-holed rubber stopper to center the microdispenser over the carbon bed.

2. Chemicals

a. 1000 ppm Hg^{2+} solution. Prepared from mercuric chloride (Matheson, Coleman and Bell), deionized distilled water and conc. HNO_3 as described in Chapter 1.

b. 45-60 Mesh Chromosorb W

c. 45-60 Mesh Chromosorb W with 5% (W/W) SE-30

3. Sample Collection and Analysis

a. Breath

Before any breath samples were collected, the carbon bed was heated to about 1500°C to clean off any adsorbed mercury.

The bed was heated until the recorder pen returned to 100% T. The radiofrequency (rf) generator was turned off and the bed allowed to cool under air or scrubbed nitrogen for 5 minutes. A breath sample was then collected by having the subject hold the end of the Tygon tubing in his mouth. He breathed in through his nose and exhaled through the tubing directly onto the unheated carbon bed. The atomizer cell vacuum pump pulled the exhaled air over the carbon bed at a rate of 200 mL/min. The ten-minute sampling period collected 2 liters of exhaled breath.

After the sample had been collected, 30 seconds were allowed for the breath remaining in the tubing to be pulled onto the bed. The flow of air was diverted around the atomizer by means of a bypass valve. The rf generator was turned on and the bed was heated to decompose and atomize the trapped sample. Thirty-five seconds were allowed for the heating and atomization process before air (or nitrogen) flow was resumed through the cell. The atomized sample was drawn through the light path where atomic absorption took place.

For resonance line absorption measurements (253.7 or 184.9 nm), the demountable Hg hollow cathode lamp was used. For measurement of molecular background, the sample collection and analysis procedure was repeated, but a deuterium lamp was used in place of the hollow cathode lamp. Background signals from air, nitrogen and breath samples were measured on at least five different occasions. No background absorption was ever seen at

253.7 nm. Background absorption signals measured at 184.9 nm were due to H_2 and CO formed during the atomization process.

All stages of the sample collection procedure were timed using a stopwatch so that samples could be collected and analyzed in a reproducible manner.

A "blank" sample was collected and analyzed to determine the absorption from air or nitrogen pulled through the atomizer in between breath samples. A two liter aliquot of air or nitrogen was drawn over the bed in place of a breath sample. The absorbance from the blank was subtracted from the absorbance of the samples run that day.

Breath samples were collected from adult males and females in the Louisiana State University population. Chemistry faculty, graduate students, undergraduate students and secretarial personnel consitituted the sampled group. None of the sampled population was occupationally exposed to mercury (other than normal laboratory exposure). One of the subjects was currently working with elemental, inorganic and organic mercury on a limited basis. Another subject, to be discussed below, was exposed to mercury through ingested paint. Three of the forty-two subjects were cigarette smokers. Most subjects consumed fish (a known source of mercury) two to four times per month. None of the subjects had undergone any dental work within the previous six months.

Speciation of mercury compounds in breath was attempted using two of the selective adsorption tubes described by Johnson and Braman.[234] These tubes were placed in the Tygon tubing line leading to the atomizer so that the breath sample passed through the adsorption tubes before reaching the carbon bed.

b. Saliva

Saliva samples were collected from fourteen individuals in the sample population discussed above. The subjects rinsed their mouths with tap water, swallowed a few times and expectorated into nitric acid-cleaned polyethylene vials. It was possible that rinsing with tap water could dilute the saliva and result in a negative error in the determination. This was not thought to be a serious problem and, in any case, was preferable to analyzing saliva contaminated with coffee and chewing gum. Saliva samples were analyzed immediately after collection. Two methods of analysis were used.

i. Carbon Disk Method

Carbon disks were prepared by punching 6 mm disks from sheets of pyrolytic graphite-coated Graphoil with a hole punch. The disks were cleaned by heating them in the carbon bed at 1450°C until no mercury absorption signal was seen. After the bed was cooled under scrubbed nitrogen, the disks were removed and stored in capped nitric acid-cleaned polyethylene vials. The disks were cleaned about two hours prior to use.

A 1 μL aliquot of saliva was placed on a Graphoil disk with a Hamilton microliter syringe. The disk was dropped onto the hot carbon bed and the absorption signal measured. Samples were analyzed in triplicate. Background absorption was measured with the deuterium lamp on additional aliquots of the sample.

ii. Direct Injection with Microdispenser

Two microliter aliquots of saliva were injected directly onto the hot carbon bed with the modified Drummond microdispenser. Background absorption was measured with the deuterium lamp on separate aliquots.

Identification of the chemical form of mercury in saliva was attempted. The procedure and results are discussed in Chapter 7 of this dissertation.

4. Calibration

a. Breath

Calibration was accomplished by injecting various volumes of air saturated with mercury vapor onto the cold carbon bed with a gas-tight syringe. The amount of mercury injected was calculated from vapor pressure data.[127] The adsorbed mercury vapor was analyzed as if it were from a trapped breath sample.

The sensitivity, defined as that quantity of mercury equal to 1% absorption, was $(1.5 \pm 0.2) \times 10^{-11}$ g for the 184.9 nm line and $(1.0 \pm 0.1) \times 10^{-10}$ g for the 253.7 nm line.

Calibration curves were linear up to about 70 ng Hg (approximately 3 mL of air saturated with Hg vapor) at the 253.7 nm line.

With larger volumes, an absorption signal began to be recorded before the bed was heated. This was probably due to mercury being released from the carbon near the bottom of the bed, which was warmed by the heated optical path.

b. Saliva

Aqueous solutions of Hg^{2+} in the 0.01 to 10 ppm range were prepared fresh daily by dilution of the 1000 ppm stock Hg^{2+} solution. Standards were diluted with deionized distilled water. Calibration curves were run by the two methods described for introduction of saliva samples into the atomizer, the carbon disk method and direct injection with the Drummond microdispenser. The absorbance due to blank carbon disks and deionized distilled water was measured and subtracted from the absorbance of the standards when required. Background absorption was measured with the deuterium lamp.

Calibration curves were linear up to 10 ppm Hg at the 253.7 nm line. Typical calibration curves are shown in Figures 15 and 16. Precision was determined by making 20 injections of a 2 ppm Hg solution. The mean found by the carbon disk technique was 2.0 ± 0.4 ppm (mean $\pm \sigma$); that found by direct injection was 2.0 ± 0.2 ppm (mean $\pm \sigma$).

C. RESULTS

1. Mercury Concentrations in Laboratory Air and N_2 Purge Gas

An aliquot of laboratory air or N_2 purge gas was

analyzed in order to detect any mercury in these gases which would be trapped by the carbon bed during the cooling period between breath samples. This determination was considered to be a blank for subsequent breath samples.

Absorption traces of laboratory air and nitrogen purge gas at 184.9 nm are shown in Figure 42. An absorption trace of laboratory air at 253.7 nm is shown in Figure 43. Resonance absorption and background absorption are shown.

Mercury concentrations found in laboratory air on various dates are listed in Table 30. All data were measured at 253.7 nm. The mean mercury concentration found was 2.3 μg Hg/m^3 air. The range was from 0.1 to 6.3 μg Hg/m^3 air.

2. Mercury Concentrations in Breath

Absorption traces of breath at 184.9 nm and 253.7 nm are shown in Figures 43 and 44. Use of the 184.9 nm resonance line was investigated in order to exploit the sensitivity of this line over the spin-forbidden 253.7 nm line. As can be seen in Figure 44, 100% absorption of the 184.9 nm resonance line occurred at the start of the breath sample collection period. This absorption was due to oxygen, carbon dioxide, water vapor and other molecular constituents of breath. At the end of the ten minute sampling period, nitrogen purge gas was introduced into the atomizer and transmission of the resonance line rapidly increased. However, absorption of the resonance line by mercury released from the carbon bed began before the resonance signal had returned to the

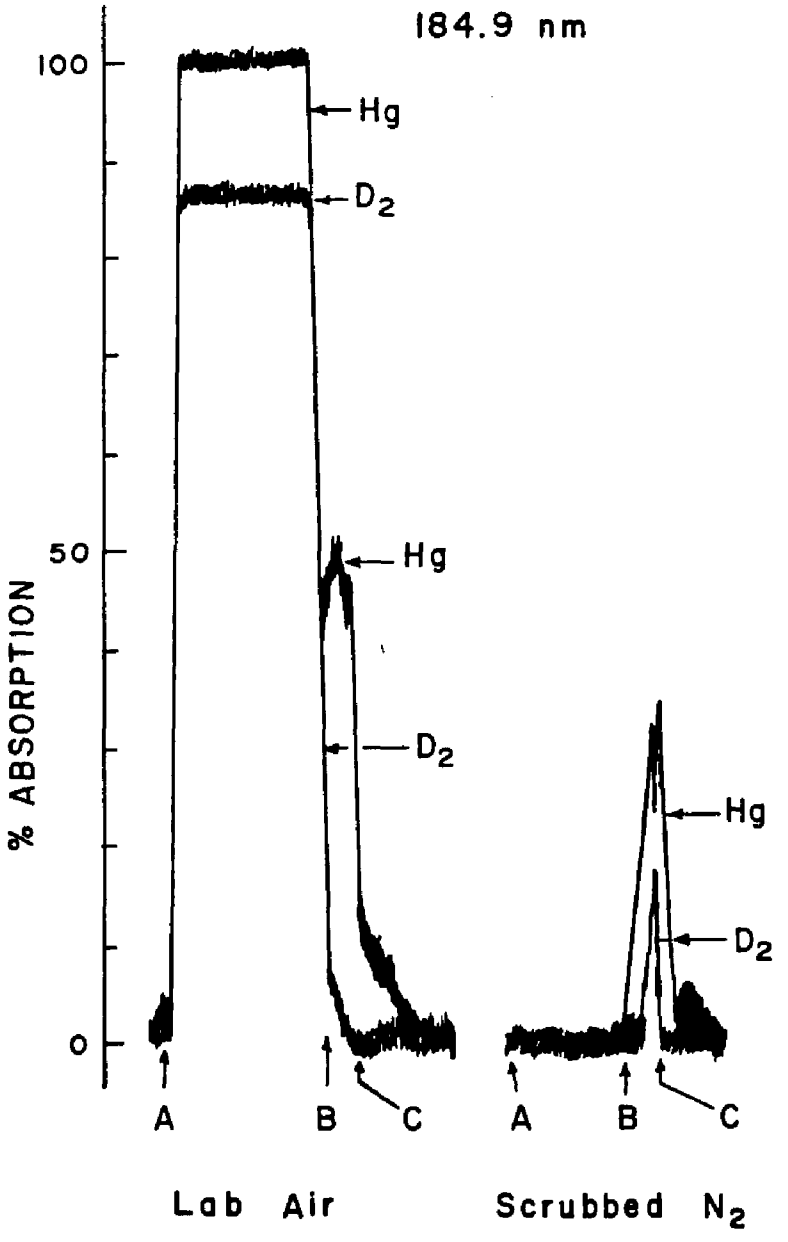


FIGURE 42: ABSORPTION TRACES OF AIR AND N_2 AT 184.9 nm. POINT A: SAMPLE INTRODUCED ONTO COLD CARBON BED. POINT B: SAMPLE FLOW STOPPED AND RF GENERATOR TURNED ON TO HEAT CARBON BED. POINT C: ATOMIZED SAMPLE PULLED INTO LIGHT PATH. RESONANCE (Hg) AND MOLECULAR BACKGROUND ABSORPTION (D_2) ARE SHOWN.

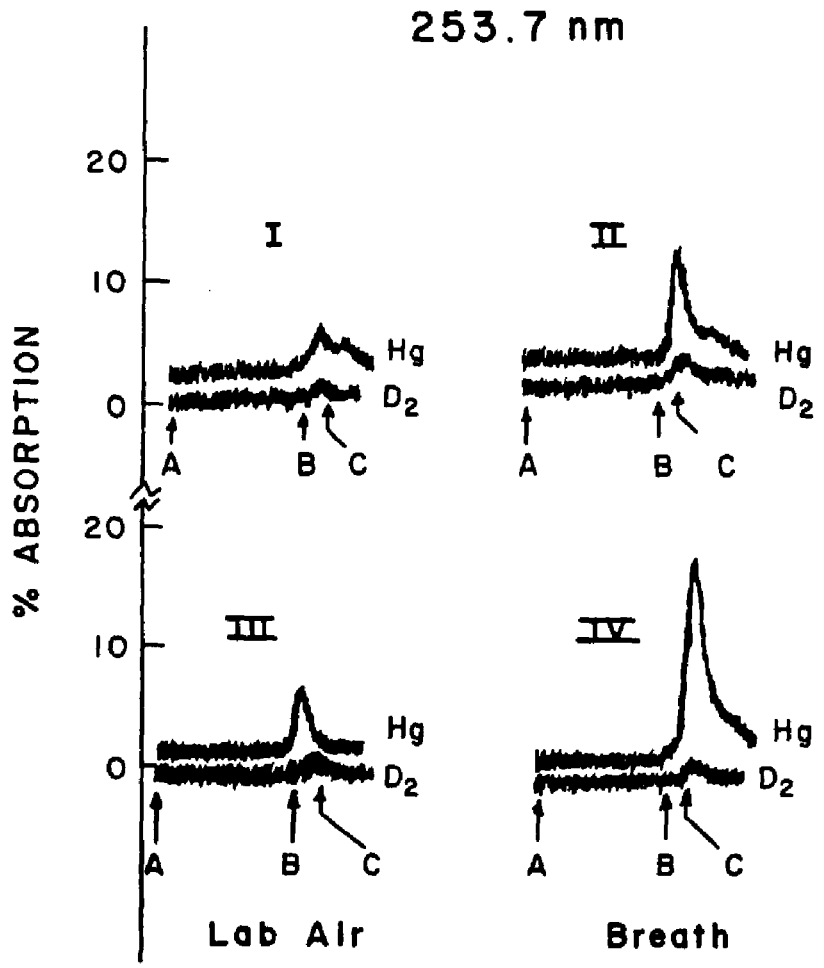


FIGURE 43: ABSORPTION TRACES OF AIR(I AND III) AND BREATH (II AND IV) SAMPLES AT 253.7 nm. POINT A: SAMPLE INTRODUCED ONTO COLD CARBON BED. POINT B: SAMPLE FLOW STOPPED AND RF GENERATOR TURNED ON TO HEAT CARBON BED. POINT C: ATOMIZED SAMPLE PULLED INTO LIGHT PATH. RESONANCE (Hg) AND MOLECULAR BACKGROUND ABSORPTION (D_2) ARE SHOWN.

Table 30

Mercury Concentrations in Laboratory Air

Date	ng Hg/2L air	µg Hg/m^3 air
07/14/81	2.6	1.3
07/15/81	1.8	0.9
07/16/81	11.0	5.5
07/20/81	8.0	4.0
07/24/81	1.4	0.7
07/31/81	4.0	2.0
08/27/81	2.0	1.0
10/26/81	1.2	0.6
10/28/81	0.3	0.1
12/14/81	3.0	1.5
12/15/81	3.0	1.5
12/15/81	11.8	5.9
12/17/81	3.9	1.9
01/18/82	8.5	4.3
01/21/82	0.7	0.3
02/02/82	12.5	6.3

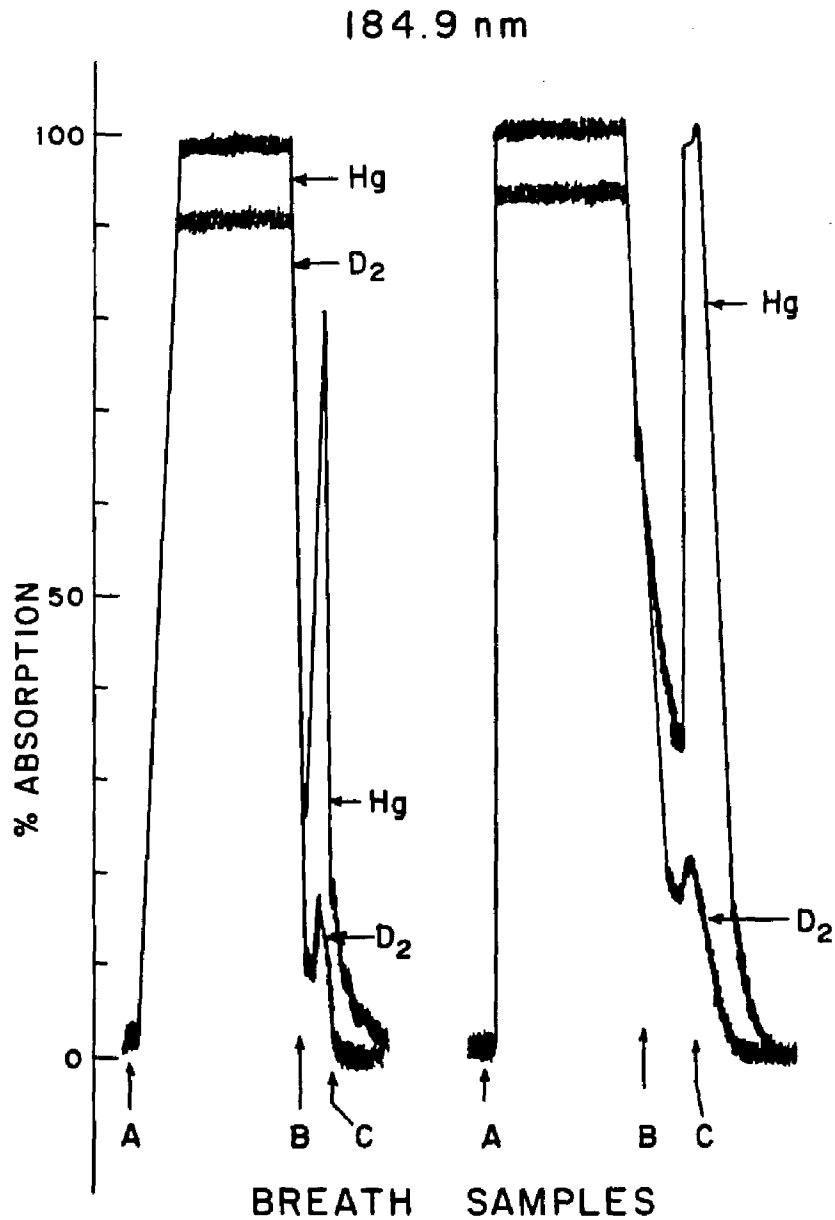


FIGURE 44: ABSORPTION TRACES OF BREATH SAMPLES AT 184.9 nm. POINT A: SAMPLE INTRODUCED ONTO COLD CARBON BED. POINT B: SAMPLE FLOW STOPPED AND RF GENERATOR TURNED ON TO HEAT CARBON BED. POINT C: ATOMIZED SAMPLE PULLED INTO LIGHT PATH. RESONANCE (Hg) AND MOLECULAR BACKGROUND ABSORPTION (D_2) ARE SHOWN.

baseline. This resulted in a mercury absorption peak with a non-horizontal baseline which made the peak height difficult to measure reproducibly. In addition, a correction factor had to be applied if the absorption signal began at less than 100% T.

No molecular absorption occurred at 253.7 nm and the sensitivity was adequate for breath analysis. Therefore, no quantitative analyses were made at 184.9 nm.

Mercury concentrations in the breath of 42 subjects were determined. All determinations were made at 253.7 nm. The results are listed in Table 31. The overall average mercury concentration in breath was found to be 2.65 ng Hg/L breath; that for males was 2.10 ng Hg/L breath and for females, 3.45 ng Hg/L breath.

3. Variation in Mercury Concentration in Breath with Exposure to Mercury

Two females were studied who had been exposed to mercury on a limited basis. The breath of the first subject was analyzed on nine different occasions over an eight month period. The mercury concentrations found ranged from <0.05 ng/L to 8.6 ng/L and are listed in Table 32. Analysis number 4 was performed about 3 hours after the subject had handled elemental mercury. Analysis number 9 was performed about two hours after the subject had weighed out solid methylmercury chloride. These analyses showed mercury concentrations in the breath of 8.6 and 6.2 ng/L, respectively which were considerably higher than concentrations

Table 31

Mercury Concentration in Breath

Female		Male	
Subject no.	ng Hg/2L Breath	Subject no.	ng Hg/2L Breath
1	3.5	21	10.4
2	1.5	22	0.5
3	3.2[a]	23	1.6
4	1.0	24	1.5
5	3.1	25	6.5
6	3.1	26	0.7
7	6.3	27	3.3
8	15.5	28	0.7
9	1.8[a]	29	none detected
10	7.2	30	3.2
11	12.2	31	3.8
12	19.4	32	0.3
13	none detected	33	0.7
14	16.7	34	1.3
15	none detected	35	1.9
16	29.7	36	3.1
17	none detected	37	1.0
18	2.0	38	7.2
19	8.5	39	18.5
20	3.6	40	5.0
		41	4.5
		42	10.0

[a]cigarette smoker

Females
n = 20
x = 6.9 ng Hg/2L Breath
σ = 9.9
range: none detected - 29.7 ng Hg/2L Breath

Males
n = 22
x = 4.2 ng Hg/2L Breath
σ = 4.4
range: none detected - 18.5 ng Hg/2L Breath

Overall
n = 42
x = 5.3 ng Hg/2L Breath
σ = 6.5
range: none detected - 29.7 ng Hg/2L Breath

Table 32

Mercury Concentrations in Breath of an Individual Exposed to Mercury

Subject 1

Analysis no.	Date	ng Hg/L Breath
1	07/14/81	none detected
2	07/16/81	1.9
3	07/31/81	none detected
4[a]	10/26/81	8.6
5	10/28/81	1.8
6	12/15/81	none detected
7	12/17/81	3.4
8	01/21/82	none detected
9[b]	02/09/82	6.2

[a]exposed to elemental mercury

[b]exposed to methylmercuric chloride

found on other occasions, which had an average value of 1.0 ng Hg/L.

The second subject was exposed to mercury through oral ingestion of mercury-containing paint. She was in the habit of pointing her paint brush with her mouth when painting ceramic figurines. The exposure was discovered when the subject was asked to donate a breath sample for analysis. The mercury concentration found was 22.2 ng Hg/L breath, the highest concentration measured in this study at that point. A repeat sample confirmed the high level. The subject then ceased putting the brush in her mouth. Mercury levels in the subject's breath on various dates are listed in Table 33.

The mercury levels found in the breath of these 2 subjects were not included in the data in Table 31.

4. Speciation of Mercury in Breath

An attempt was made to speciate mercury in breath through the use of the two adsorption tubes described previously. The HCl-treated SE-30-coated Chromosorb W was supposed to retain $HgCl_2$-like compounds. The NaOH-treated Chromosorb W was supposed to retain CH_3HgCl-like compounds. Elemental mercury vapor and dimethylmercury should have passed through the adsorption tubes and been retained on the carbon bed. Air saturated with mercury vapor was used to check the adsorption tube system.

1 mL of air saturated with Hg° (about 20 µg Hg) was injected through both adsorption tubes. The sample was treated as though it

Table 33

Mercury Concentrations in Breath and Saliva
of an Individual Exposed to Mercury

Subject 2

Date	ng Hg/2 Breath	ppm Hg in Saliva
12/14/81	22.2	0.80
12/15/81	14.2	----
01/18/82	32.0	----
02/09/82	9.5	----
05/05/82	5.6	0.08

Note: Exposure ceased 12/31/81

was a breath sample. No absorption signal was seen when the carbon bed was heated, compared to a 20% absorption signal for the same volume of air injected without the adsorption tubes. Ten milliliters of air saturated with mercury vapor were injected through the adsorption tubes. A 6% absorption signal was generated, compared to a 93% absorption signal without the adsorption tubes.

Each adsorption tube was tried separately, with the same results. The tubes obviously adsorbed elemental mercury vapor, although they were reported to not adsorb it. The method was therefore unsuitable for the speciation of mercury compounds in breath.

5. Mercury Concentrations in Saliva

Mercury was determined in the saliva of fifteen subjects, most of them from the breath-sampled population. The two mercury exposed subjects were included in the sample group.

Results are listed in Table 34. The overall mean concentration was 0.27 ppm Hg with a standard deviation of 0.11 and a range of 0.15 - 0.50 ppm Hg. The paint-exposed individual had a saliva mercury level of 0.80 ppm. This was greater than 4σ from the mean and therefore was not included in calculating the mean. Her saliva mercury level dropped with time, as can be seen in Table 33.

The female sample population was too small to allow statistical comparison of the average concentrations of mercury in the

Table 34

Mercury Concentrations in Saliva

Female		Male	
Subject no.	ppm Hg	Subject no.	ppm Hg
1	0.32	5	0.24
2[a]	0.39	6	0.19
3	0.32	7	0.21
4[b]	0.80	8	0.1
4[c]	0.08	9	0.5
		10	0.28
		11	0.21
		12	0.15
		13	0.10
		14	0.40

[a]exposed to elemental and organic mercury

[b]exposed to mercury through ingestion of paint-sample taken 12/14/81

[c]same subject as 4[b]--sample taken 5/5/82

saliva of the male and female populations.

2 μL saliva samples gave resonance line absorption signals of 2-8% with no molecular background absorption.

6. Correlation Among Mercury Concentrations in Breath, Saliva and Hair

The mercury in both breath and saliva of twelve subjects were measured in this study. Sixteen of the breath-sampled population had mercury levels in scalp hair determined in a earlier study, which was reported in Chapter 5 of this dissertation. Nine subjects had data available for all three matrices.

Correlation diagrams for mercury concentrations in these matrices are given in Figures 45-47.

D. DISCUSSION

1. Advantages of the Quartz "T" Atomizer

The use of the quartz "T" atomizer in the determination of mercury in breath and saliva has several advantages over other methods of analysis:

a. It permitted use of the more sensitive 184.9 nm rsonance line as well as the more commonly used 253.7 nm line.

b. It eliminated the need for sample pretreatment, due to the efficient one-step atomization process. Atomization took place outside of the light path, so that scatter and background absorption were decreased.

c. Mercury from breath samples was trapped directly in the atomizer, so that no transfer of the trapped sample was

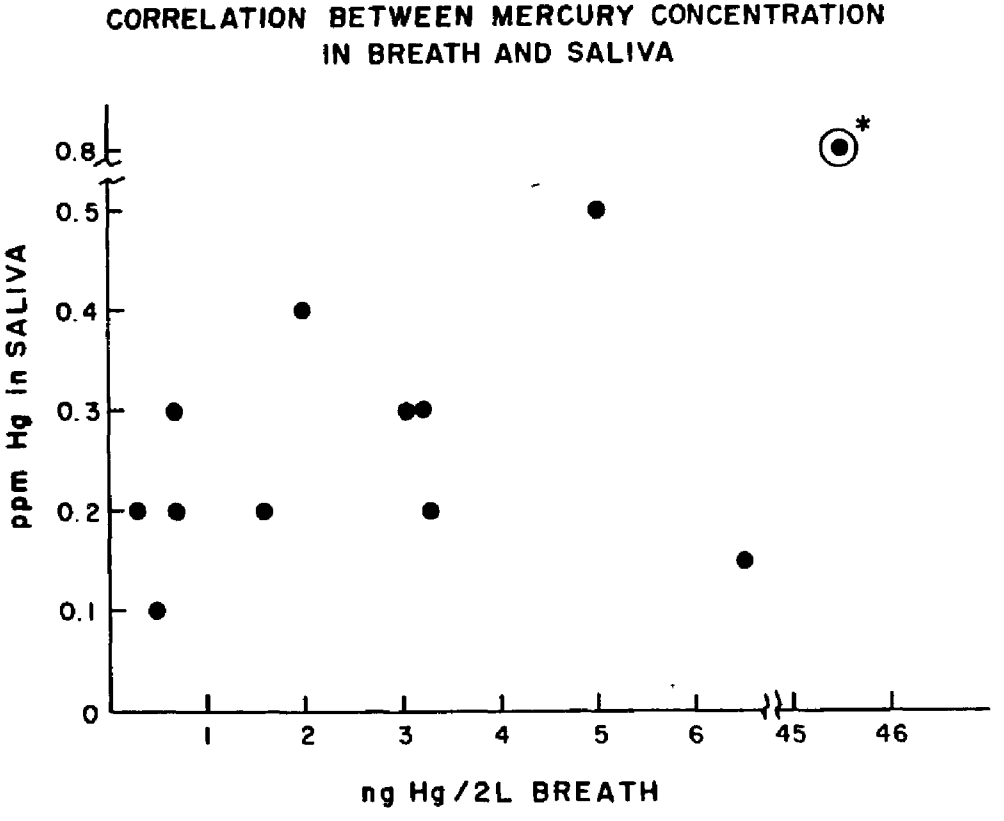


FIGURE 45: NO CORRELATION EXISTED BETWEEN MERCURY LEVELS IN BREATH AND IN SALIVA FOR A NORMAL POPULATION. (CORRELATION COEFFICIENT = 0.244, EXCLUDING POINT MARKED WITH ASTERISK). RECENT EXPOSURE TO MERCURY THROUGH INGESTION OF MERCURY-CONTAINING PAINT (POINT MARKED WITH ASTERISK) RESULTED IN VERY HIGH LEVELS OF MERCURY IN BOTH BREATH AND SALIVA.

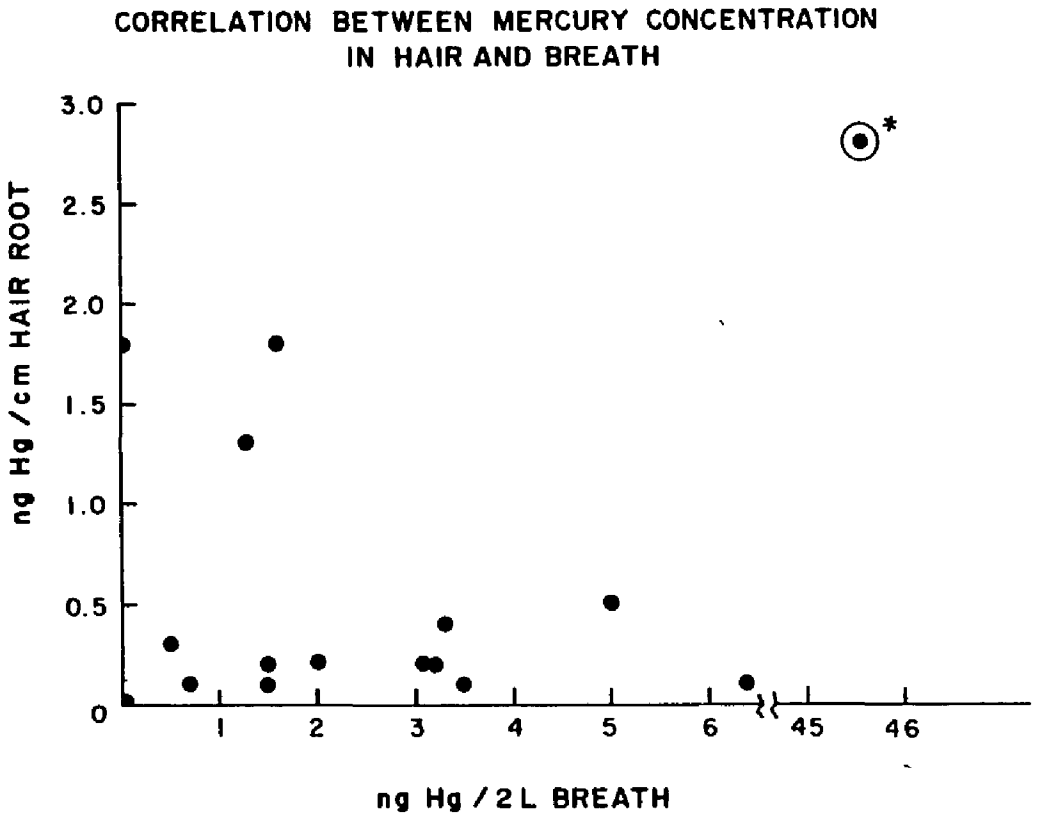


FIGURE 46: NO CORRELATION EXISTED BETWEEN MERCURY LEVELS IN HAIR AND IN BREATH FOR A NORMAL POPULATION. (CORRELATION COEFFICIENT= -0.292 , EXCLUDING POINT MARKED WITH ASTERISK). RECENT EXPOSURE TO MERCURY THROUGH INGESTION OF MERCURY-CONTAINING PAINT (POINT MARKED WITH ASTERISK) RESULTED IN VERY HIGH LEVELS OF MERCURY IN BOTH HAIR AND BREATH.

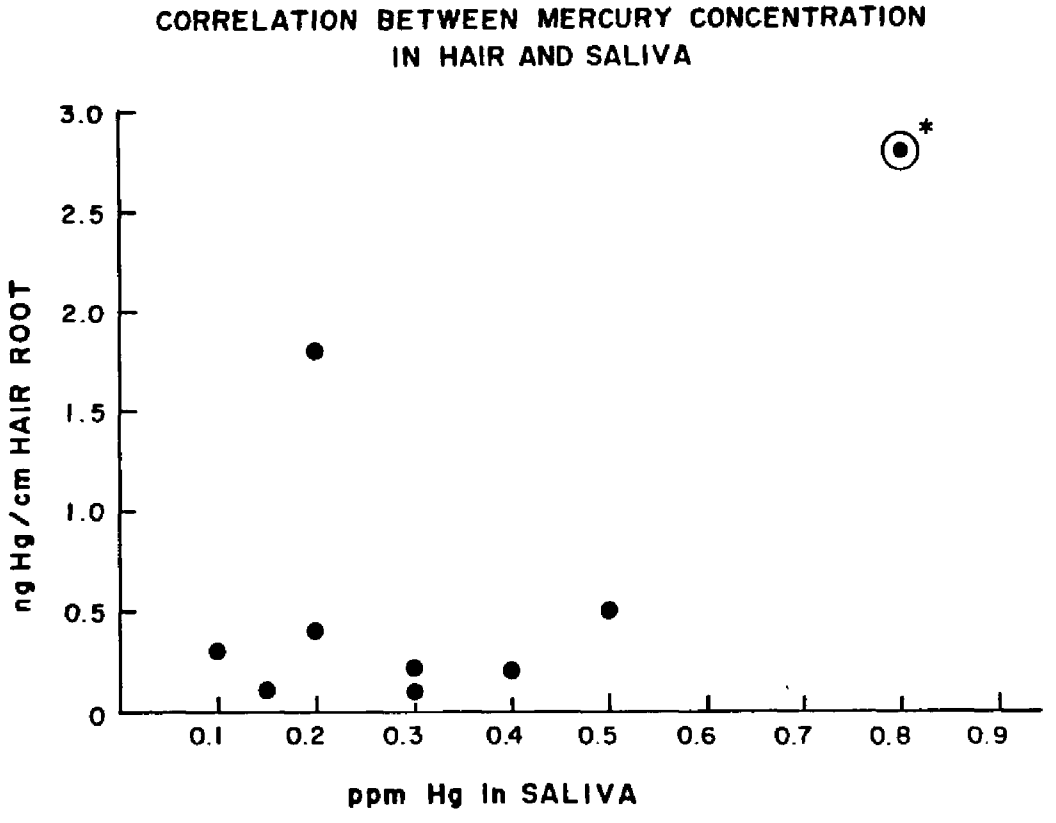


FIGURE 47: NO CORRELATION EXISTED BETWEEN MERCURY LEVELS IN HAIR AND IN SALIVA FOR A NORMAL POPULATION. (CORRELATION COEFFICIENT = -0.119, EXCLUDING POINT MARKED WITH ASTERISK). RECENT EXPOSURE TO MERCURY THROUGH INGESTION OF MERCURY-CONTAINING PAINT(POINT MARKED WITH ASTERISK) RESULTED IN VERY HIGH LEVELS OF MERCURY IN BOTH HAIR AND SALIVA.

necessary. This eliminated one possible source of negative error.

d. Accuracy was improved for saliva analyses because the direct determination eliminated losses of mercury due to incomplete recovery from concentration steps. In addition, no mercury contamination occurred from reagents normally used for wet ashing.

e. Accuracy for breath and saliva analyses was improved because all of the mercury which entered the atomizer was passed through the light path and was detected. No losses such as those due to volatilization during the drying and ashing cycles of commercial graphite furnaces or due to incomplete reduction of mercury during cold-vapor analysis could occur.

2. Analysis of Breath Using the 184.9 nm Resonance Line

As can be seen in Figure 44, the mercury absorption peak occurred on the shoulder of the molecular background absorption. It is clear from the absorption trace that mercury was indeed present in expired air, but it was not known if any volatile mercury compound was eluting from the carbon under the molecular absorption peak. Since the 253.7 nm resonance line proved to have sufficient sensitivity for breath analysis without the problems of molecular absorption, no further work was done at 184.9 nm.

3. Analysis of Breath Using the 253.7 nm Resonance Line

The sensitivity of the 253.7 nm line (defined as a 1% absorption signal) was about 1×10^{-10} g Hg. This was perfectly adequate for breath analysis, since most breath samples

gave an absorption signal of between 3% and 30% absorption. Molecular absorption, determined by the absorption signal of a sample measured with the deuterium lamp, was negligible, as can be seen in Figure 43.

4. Range of Mercury Concentrations in Breath of a "Normal" Population

No studies of mercury concentrations in the breath of a normal population were found in the literature. It is not unreasonable to suppose that breath is a means of excretion of mercury from the body, in a manner analogous to excretion of Se and Te. It must be remembered that possible sources of mercury in the sample collected include mercury volatilized from the lung, mercury released from the surface of dental amalgam fillings in the mouth, mercury volatilized by bacteria in the mouth, mercury volatilized from saliva or entrained in saliva droplets in the exhaled breath.

The average mercury concentration in the breath of the population sampled was found to be 2.65 ng Hg/L breath or 2.65 $\mu g/m^3$. It has been estimated that a person exhales 10^4 L breath/day.[8] This would result in excretion of 26.5 µg Hg/day through the breath.

A significant difference exists between the mean concentrations of mercury in breath for the male and female populations sampled.

5. Variations in Mercury Concentration in Breath on Exposure to Mercury

a. Subject 1

This subject had an average mercury level of 1.0 ng Hg/L breath as determined by seven analyses over a period of eight months. On two occasions, mercury levels significantly higher than this were found.

After a morning was spent in preparation of elemental mercury-copper foil amalgams, a value of 8.6 ng Hg/L breath was measured. Exposure was to Hg°, primarily through inhalation, although some absorption through the skin may have occurred. On the second occasion, the subject had weighed out solid CH_3HgCl for preparation of solutions. Exposure was again primarily through inhalation of fine dust with possible minor skin contact. A breath sample taken afterward contained 6.2 ng Hg/L breath.

b. Subject 2

This subject was first sampled on 12/14/81 and was found to have a very high level mercury in her breath, 22.2 ng/L. She did not smoke, had had no recent dental work and consumed fish about four times per month. She explained that for about one month prior to the sampling data she had been painting ceramic ornaments and had been pointing the paint brush with her mouth. Three of the ceramic paints (black, white, and gray) were analyzed and were found to contain mercury. The chemical form of the mercury was not known. The subject refrained from putting the

paint brush in her mouth from this time on and exposure ended about two weeks later. The subject's breath was monitored for two months and the mercury level showed a decrease by the second month. A sample taken 5 months after exposure had a level of 5.6 ng Hg/L, well within the "normal" range.

The subject's husband also had a breath sample analyzed, but the concentration was found to be within one standard deviation of the average concentration. It seemed, therefore, that the high mercury levels founds in Subject 2's breath were due to her paint exposure and not to an exposure common to her and her spouse, e.g., diet, residence, etc. Moreover, saliva samples from Subject 2 and her husband showed that she had the highest mercury concentration of the population sampled, while his value was within 2σ of the normal mean.

6. Attempt at Speciation of Mercury in Breath

The chemical form of mercury in the breath was not known. Previous studies of animals and man indicated that mercury can be exhaled as Hg° and $(CH_3)_2Hg$, but these studies were done after deliberate exposure to mercury. The chemical form of at least some exhaled mercury was the same as the chemical form to which the subject was exposed.

Since several elements can be volatilized through the lungs as dimethyl compounds (e.g., $(CH_3)_2Se$, $(CH_3)_2Te$), dimethylmercury is a possible form of exhaled mercury. Elemental mercury is also a possible form of exhaled mercury and may also be

present in breath due to amalgam dental fillings.

It is of great interest to note that researchers have found mercaptans and dimethylsulfide in breath.[236,237] These are assumed to be metabolic products from sulfur-containing amino acids. The mobile forms of mercury *in vivo* are thought to be mercury-gluthathione and mercury-cysteine compounds.[238,239] It is possible that mercury may be volatilized as a mercury-sulfur compound.

An attempt was made to use a speciation technique reported in the literature[234] which had separated $HgCl_2$, CH_3HgCl, Hg° and $(CH_3)_2Hg$ in air by selective adsorption. The first two adsorption tubes in the sampling train were reported not to adsorb Hg° or $(CH_3)_2Hg$. Accordingly, if the only chemical forms of mercury in breath were Hg° and/or $(CH_3)_2$ the absorption signal from breath should be unchanged on passing through these adsorption tubes.

Both tubes, one containing HCl-treated SE-30-coated Chromosorb W and the other, NaOH-treated Chromosorb W, were placed in series in the Tygon tubing leading to the atomizer. However, no absorption signal was seen upon injection of air saturated with mercury vapor into the speciation train. Complete adsorption of as much as 100 ng Hg by the tubes occurred despite the report that these materials did not adsorb Hg°. Each tube was also tried separately and complete adsorption of the injected Hg° was seen for each. Therefore, speciation of mercury in breath was not

possible using this technique.

7. Range of Mercury Concentrations in Saliva

Very little work has been done on mercury levels in saliva, especially in a normal population. In the study by Joselow et al.,[231] less than 0.005 ppm Hg was found in the saliva of non-occupationally exposed subjects, but in this case saliva was collected from the parotid gland with a suction device, so that saliva did not contact the rest of the mouth.

The average mercury level found in saliva in this study was 0.27 ppm. This value is significantly higher than that reported by Joselow et al.[231] The higher value could be the result of the improved accuracy of our direct determination. It could also result from increased mercury concentration in whole saliva through contact with amalgam fillings and bacteria in the mouth. All of the subjects had some amalgam fillings but the exact number was not ascertained.

The saliva mercury level of the paint-exposed individual was greater than 4σ from the mean of the normal population. It was evident that saliva mercury levels did reflect recent mercury exposure, confirming the reports of Joselow et al.[231] and Stofford.[220] The mercury level in her saliva decreased to normal levels over a six-month period following the last exposure. The other female with the two high mercury values in breath had a mercury level in saliva of 0.39 ppm, well within 2σ of the mean. Her breath was measured at the same time the saliva sample was

taken and was found to have no detectable mercury.

8. Correlation of Mercury Levels in Breath, Saliva and Scalp Hair

As can be seen in Figures 45-47, no correlation existed among mercury levels in breath, saliva and scalp hair for a normal population. In light of the findings of Joselow et al.[231] that mercury levels in saliva appeared to reflect mercury levels in blood, it is somewhat surprising that there is no correlation between mercury levels in saliva and in breath. Breath should reflect mercury levels in blood if Hg^{2+} is the major form of mercury in blood and and equilibrium between Hg^{2+} and Hg° is present in blood. Therefore, mercury in breath should be correlated with mercury in saliva. It is possible (and probable) that mercury does not exist as Hg^{2+} in the blood, but as a complex with glutathione or cysteine. It is also possible that the major part of mercury in breath does not come from the lungs, but from amalgam fillings in the mouth.

A definite correlation exists for mercury levels in all three matrices for recent mercury exposure. The paint-exposed individual had the highest levels in breath, saliva and scalp hair of all subjects surveyed. The breath and saliva levels both dropped back to "normal" within six months of the last exposure.

9. Daily Excretion of Mercury from the Body

From the data accumulated in the studies reported in this dissertation, it was possible to estimate the daily excretion of

mercury from the body. The average concentrations of mercury in urine, sweat, hair and breath can be multiplied by the amount of each matrix excreted per day to obtain the total amount excreted, as follows:

Urine: 1.3 ppm Hg x 1.5 L/day = 2 mg Hg/day

Sweat: 0.5 ppm Hg x 3.0 L/day = 1.5 mg Hg/day

Hair: 1.3×10^{-5} mg/m x 40 m/day = 0.5 μg Hg/day

Breath: 2.65 g Hg/m^3 x 10 m^3/day = 26.5 μg Hg/day

The total amount of mercury excreted per day can therefore be estimated to be the sum of the above figures, 3.53 mg Hg/day. Excretion through saliva was not included in these calculations, because most saliva was reswallowed and the mercury reabsorbed.

It was evident from the data obtained in these studies that urine and sweat were equally important routes of excretion while breath and hair played minor roles. Other modes of elimination which were not investigated in these studies were faces (major route of excretion) and nail tissue, exfoliated skin, tears, and milk (minor routes of excretion).

The estimated amount of mercury excreted daily, 3.53 mg, exceeded the literature estimates of daily mercury intake, 50 μg Hg.[2,10] This was not surprising. Much of the data on mercury concentrations in food were obtained by gravimetric methods in the 1940's[2,10] and were most probably too low. Since the levels

of mercury found in water and biological samples in this study were about 10^2 higher than literature values, it was reasonable to assume that total intake of Hg is about 50 µg Hg x 10^2 per day, or 5 mg Hg/day. This would indicate that excretion and intake are of the same order of magnitude, which would support the reported values[2,10] for the half-life of mercury in the human body of 58-190 days.[12-20]

E. CONCLUSIONS AND SUMMARY

a. The use of the quartz T atomizer for atomic absorption spectroscopy enabled the development of a simple, sensitive, effective method for the determination of mercury in breath and saliva.

b. The average mercury concentration in the breath of a normal adult population was found to be 2.65 µg/m^3 breath.

c. The average mercury concentration in the saliva of a normal adult population was found to be 0.27 ppm Hg.

d. Breath and saliva were shown to reflect recent exposure to several chemical forms of mercury. Therefore, both matrices can serve as biological indicators of mercury exposure.

e. No correlation was found among mercury levels in breath, saliva and scalp hair for a normal population. A definite correlation appeared to exist among mercury levels in all three matrices for recent mercury exposure.

f. Approximately 3.53 mg Hg/day was excreted by the body in urine, sweat, hair and breath.

PART II

SPECIATION OF MERCURY COMPOUNDS

CHAPTER 7

SPECIATION OF MERCURY COMPOUNDS BY DIFFERENTIAL VOLATILIZATION-ATOMIC ABSORPTION SPECTROSCOPY

A. INTRODUCTION

The toxicity of mercury depends on both its concentration and chemical form. The chemical form of mercury in the body controls its absorption, transport, retention and excretion. In order to elucidate the metabolic pathways followed by mercury in the body, it is necessary to be able to identify mercury compounds at trace levels in complex biological matrices.

1. Current Methods for Mercury Speciation

Many procedures for metal speciation involved a separation step using a chromatographic or solvent extraction technique[240] followed by determination of the metal content of each fraction.

The application of chromatographic techniques to the speciation of mercury compounds has been reviewed by Fishbein.[40,41] Every imaginable chromatographic technique has been used for mercury speciation: gas chromatography, thin-layer chromatography, paper chromatography, liquid chromatography, ion-exchange chromatography, gel filtration, electrophoresis and others. These techniques have met with varying degrees of success in the differentiation of alkyl, aryl and inorganic mercury compounds.

Gas chromatography (GC) with an electron capture detector (GC-ECD) was the most common method for speciation of mercury in biological and environmental samples.[5] Many variations on the technique have appeared since the original work by Westoo,[34,83,84] but the general procedure was as follows. Hydrochloric acid was added to homogenized samples. The alkylmercuric chlorides were liberated and extracted into benzene. The benzene was back-extracted with water containing cysteine to form an aqueous mercury-cysteine complex. The aqueous layer was again acidified with HCl and re-extracted with benzene. The benzene layer was submitted to gas chromatography and the chloride attached to the alkylmercuric moiety was detected with an electron capture detector.

Mushak, Zarnegar and coworkers[119,241] determined inorganic mercury in water, blood, urine and tissues by gas chromatography with an electron capture detector. The inorganic mercury was converted chemically to alkyl or aryl mercury compounds and treated as described above. Cappon and Smith[121] developed a GC procedure for the determination of organic and inorganic mercury in biological matrices by first extracting the organomercury compounds as described above and then methylating the unextracted inorganic mercury with tetramethyl tin.

Substoichiometric isotope dilution has been used to determine total mercury and methylmercury in digested hair samples.[242] Isotope exchange methods have been used to determine inorganic

mercury in the presence of organic mercury in biologicals.[243]

Coupled chromatography-atomic spectroscopy techniques have been used to speciate mercury compounds. These had an advantage over GC-ECD techniques in that the detector (atomic absorption, atomic emission, atomic fluorescence spectrometer) was specific for mercury, unlike the ECD which detected the halide anion of alkylmercuric halides. Gonzalez and Ross[244] and Longbottom[245] used GC-AAS to separate mercury compounds. The GC effluent was burned and the mercury vapor pulled through a CV-AAS system. Ion exchange-AAS has been used to speciate mercury in water at the ppb level.[246]

Speciation of volatile mercury compounds and elemental mercury vapor in air has been accomplished through the use of selective adsorption tubes and AAS.[247] A sampling train consisting of a filter, two different GC packing materials, silver-coated beads and gold-coated beads was used selectively to retain mercury compounds. The adsorption tubes were then heated to release the mercury and the vapor was pulled through a CV-AAS system.

A number of researchers have performed selective AAS using variations of the CV-AAS technique.[156,161,188,248,249] These variations consisted of changes in the reducing conditions to allow different forms of mercury to be released from solution.

These techniques inevitably required elaborate chemical pretreatment of the sample: digestion, separation, clean-up, and

concentration steps were necessary in most of these methods. This pretreatment can cause contamination of the sample, loss of the analytes and changes in the chemical nature of the species present. Indeed, most methods of "speciation" of inorganic and organic mercury by GC and AAS merely differentiated between Hg^{2+} and RHg^{+} because the digestion procedure which was used caused RHg^{+}, which was originally bound to some constituent in the biological material, to form RHgX (X = halide ion).[250] These methods did not identify the exact chemical form of bound mercurials. Loss of volatile mercury compounds during sample handling and storage can lead to substantial errors.

The development of a direct spectroscopic method which would accomplish both separation and detection of mercury compounds would circumvent the problems encountered in sample pretreatment.

2. Differential Volatilization Theory

It was well-known that atomic absorption and atomic emission spectroscopy were subject to chemical interference, i.e., differences in volatilization temperatures and atomization efficiency for different combined forms of an element. Volatilization behavior can be classified in three catagories:[58]

a. Congruent evaporation of a molecular entity at T_1, e.g.,

$$PbCl_2(l) \rightarrow PbCl_2(g) \qquad (9)$$

b. Congruent evaporation with dissociation at T_2, e.g.

$$2CdO(s) \rightarrow 2Cd(g) + O_2(g) \qquad (10)$$

c. Incongruent evaporation, e.g.

$$CdCO_3(s) \rightarrow CdO(s) + CO_2(g) \text{ at } T_3 \quad (11)$$

followed by

$$2CdO(s) \rightarrow 2Cd(g) + O_2 \text{ at } T_4 \quad (12)$$

where $T_3 \leq T_4$. The volatilization temperature (T_1,T_2,T_3,T_4) or appearance temperature was determined both by thermodynamic properties such as vapor pressure, phase transition temperature and decomposition temperature and by the kinetics of vaporization and mass transfer.[58]

It has long been the goal of analytical chemists to eliminate these differences in volatilization, either by complexing the metal so that all atoms are in the same chemical form or by adjusting the atomizer design or conditions such that all species would be atomized with equal efficiency. It seemed, however, that the existence of these chemical interferences could provide a means for identifying various chemical forms by taking advantage of differences in volatilization temperature.

The technique required a variable-temperature first stage for volatilization of metal compounds at their vaporization temperature, a second stage capable of atomizing compounds volatilized from the first stage and an atomic spectroscopy system for detection.

Vaporization of pure compounds into an argon microwave-induced plasma was used by Hanamura[251] to show the feasibility of the technique. Bauer and Natush[58] used a temperature-

programmed furnace and a helium microwave induced plasma to speciate pure solid Cd, Hg, Pb, and Zn salts. Robinson and Rhodes[252] used a dual stage carbon atomizer to speciate Cd and Pb compounds by atomic absorption spectroscopy. Robinson and Weiss[253] demonstrated the ability to speciate Pb and Cd compounds in aqueous solution and in biological fluids by atomic absorption spectroscopy employing a variable temperature platinum loop as a first stage and an inductively heated carbon bed atomizer, the quartz "T", as the second stage.

For the initial experimentation with such a technique for mercury speciation, it was decided to use a wire loop as the first stage of the system. Such an apparatus had been used before with the quartz "T" atomizer, which would serve as the second stage. A heat stable and inert (non-amalgamating) metal was necessary for fabrication of the wire loop. Platinum was the material chosen.

3. The Use of Wire Loops in AAS

Electrically heated wire loops have been used as atomizers for atomic spectroscopy by a number of researchers. Wire loops made of tungsten and of platinum[254] and of platinum-rhodium alloy[255] have been suggested for use as atomizers for atomic fluorescence spectroscopy. Wire filaments have been used to preconcentrate metals from urine prior to atomization.[256] Tungsten and tungsten alloy wire loops have been employed as atomizers for atomic absorption spectroscopy,[257-259] as have

platinum loop atomizers.[125,253,260] In these studies, samples were pipetted onto, or electrolytically deposited onto the wire. The loops were then positioned in the optical path of the spectrometer and a voltage, usually in the form of a linear ramp, was applied to heat the loop. Absorption, emission or fluorescence was monitored as a function of voltage. The main use of these devices was to separate the analyte peak from the matrix peak, not to speciate metal-containing compounds.

In the study described in this dissertation a platinum loop has been used for both sample introduction and sample volatilization. The apparatus was quite unrefined and was designed simply to demonstrate the potential of the technique in distinguishing between mercury species. The study was qualitative rather than quantitative and served as an indication of the type of information which could be obtained by such a technique. In addition to the platinum loop system, speciation of mercury compounds by convective heating of a glass coil positioned over the quartz "T" atomizer was investigated.

B. EXPERIMENTAL

1. Equipment

The components of the atomic absorption spectrometer were the same as described in Chapter 1. The top of the quartz "T" atomizer was fitted with either the platinum loop apparatus (Figure 48) or the glass rod (Figure 49) for these studies.

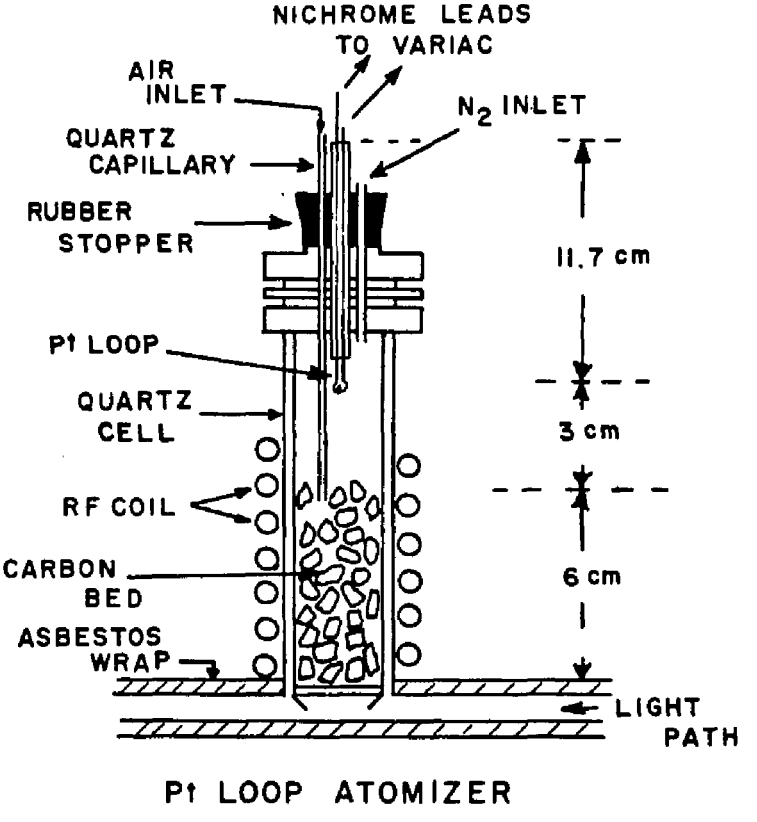


FIGURE 48: DIAGRAM OF THE PLATINUM LOOP APPARATUS FOR THE SELECTIVE VOLATILIZATION OF MERCURY COMPOUNDS, SHOWN IN PLACE IN THE QUARTZ "T".

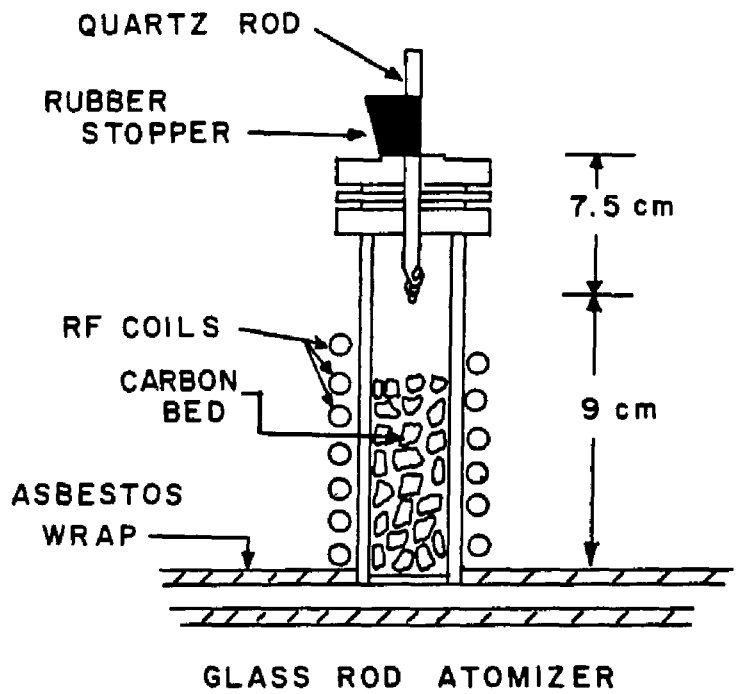


FIGURE 49: DIAGRAM OF THE GLASS ROD VOLATILIZATION STAGE SHOWN IN PLACE IN THE QUARTZ "T" ATOMIZER.

a. Platinum loop apparatus: a loop of platinum wire (0.005 inch diameter) was spot welded to nichrome wire leads which had been threaded through a ceramic tube for insulation. This assembly was held approximately 3 cm above the surface of the carbon bed by means of a rubber stopper which fitted into the opening of the stainless steel atomizer head. The stopper also held a capillary quartz tube which extended to the carbon bed surface and a quartz tube which extended only 0.5 cm below the stopper. The capillary tube was open to the air and served to introduce oxygen below the sample at the level of the carbon bed. This oxygen was necessary for efficient atomization in the bed. The point of entry avoided burning the sample on the wire loop. The shorter inlet tube was connected to scrubbed nitrogen gas and provided an inert atmosphere around the wire loop. Voltage was supplied by a Variac (Type V5) connected to a 5 volt step-down filament transformer (Stancor No. P-6432). A schematic diagram of the wire loop circuit is shown in Figure 50.

A later modification was made to the Pt loop system when it became apparent that the temperature control within the atomizer was not adequate. A glass sleeve was made to hold the loop outside of the atomizer (Figure 51).

b. Glass rod apparatus: a 15 cm length of quartz tubing (1/4 inch O.D.) was heat-sealed and drawn into a small coil at one end. The coiled end of the rod was held approximately 3 cm above the carbon bed by half of a one-holed rubber stopper fitted into the opening of the stainless steel atomizer head. Only half of a

Schematic Diagram of the Pt Wire Loop Circuit

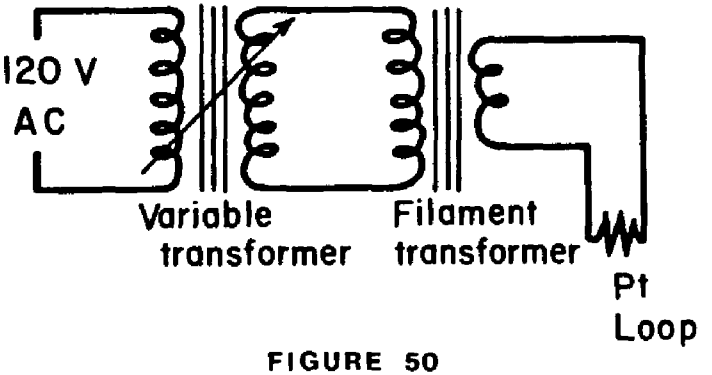
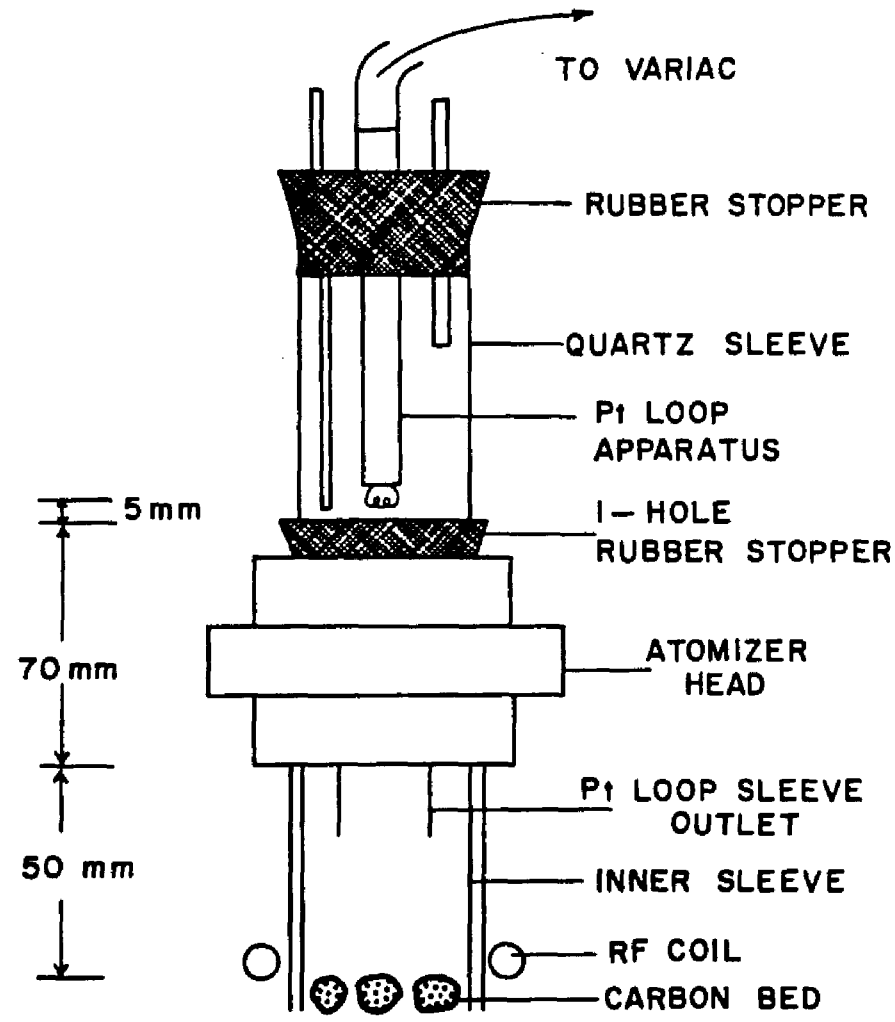


FIGURE 50



Pt LOOP OUTSIDE OF THE QUARTZ "T" ATOMIZER

FIGURE 51: DIAGRAM OF THE Pt LOOP PLACED OUTSIDE THE QUARTZ "T" ATOMIZER IN AN ATTEMPT TO ACHIEVE FINER TEMPERATURE CONTROL BETWEEN 25-250° C.

stopper was used to allow air to enter the atomizer to ensure efficient atomization (Figure 49). Other equipment utilized in these studies has been described in Chapter 1. The 253.7 nm and 184.9 nm mercury resonance lines were used in this study. A deuterium lamp was used to measure molecular background absorption.

2. Chemicals

Aqueous solutions of the following compounds were prepared: $HgCl_2$, Hg_2Cl_2, $HgBr_2$, HgI_2, $HgSO_4$, $Hg_2(NO_3)_2$, $Hg(C_2H_3O)_2$, CH_3HgCl, $(C_6H_5)_2Hg$, and parahydroxyphenyl HgCl. Soluble salts were prepared in 100 ppm Hg solutions. Saturated solutions of the insoluble salts were prepared by mixing excess salt with 25 mL water and allowing the solutions to stand for 48 hours at room temperature. The mercuric acetate solution was prepared fresh because it easily decomposed. Commercial "Tincture of Merthiolate" (1:1000 solution of "Thimerosal" [Lilly] in ethanol) was also used.

A 1000 ppm solution of EDTA was prepared from the disodium salt. A 10 mM solution of L-cysteine in KOH was prepared. A saline solution containing 2000 mg Na^+ and 20 mg K^+ per 100 mL was prepared from NaCl and KCl to approximate the ionic strength and matrix of sweat and urine. All chemicals used were of reagent-grade quality and were prepared in deionized distilled water. Distilled elemental mercury was used for calibration.

All polyethylene vials used for solutions or for sample

collection were soaked in nitric acid and were well-rinsed. Whole blood samples were collected in Vacutainers with EDTA as the anticoagulant.

3. Analytical Procedure

Prior to analysis, both the carbon bed and platinum loop were cleaned by heating in air to 1500°C. The coiled tip of the glass rod was heated in a Bunsen burner flame to clean it. The loop and rod were then allowed to cool to room temperature to receive the sample. The carbon atomizer bed was kept hot throughout.

a. Platinum loop

1 μL samples were pipetted onto the cooled loop with a 10 μL syringe (Hamilton #702-SN). The surface tension of the liquid was sufficient to hold the sample drop on the loop. The loop apparatus was then placed on top of the atomizer and gradually heated by increasing the voltage across the filament. The Variac setting was increased manually from 0 to 90 units by advancing the dial 5 units every 10 seconds. The platinum loop increased in temperature from ambient conditions to approximately 1500°C during this procedure.

During heating of the platinum wire, a flow of nitrogen (250 mL/min) was maintained through the inlet which terminated near the loop. This maintained an inert atmosphere around the loop which helped to preserve the chemical form of the metal in the sample during the heating process. Earlier investigations of cadmium and

lead[253] using this technique indicated that in the presence of air, most metal compounds were converted to the oxides and gave the same absorption traces. The inert nitrogen atmosphere prevented oxide formation.

Successive samples were analyzed by cooling the loop and removing it from the atomizer head to introduce another sample.

b. Glass rod

1 μL samples were pipetted onto the cooled coil using a 10 μL syringe. The rod was positioned at the top of the atomizer head and allowed to heat by conduction from the heated carbon bed. The glass rod remained in position until no further absorption peaks from the sample were observed. This usually required less than 10 minutes. Successive samples were analyzed by removing the rod, placing the coil in a Bunsen burner flame to clean it and allowing it to cool to room temperature before pipetting another aliquot.

C. RESULTS

The absorption spectra obtained using this technique may best be described as temperature-absorption traces. Theoretically, different forms of the metal of interest would vaporize off the loop or rod at different temperatures. Ideally, each metal compound should yield different traces with peaks occurring at characteristic temperatures.

Absorption traces were obtained using the described techniques for solutions of the previously listed pure mercury

compounds, for solutions containing mercuric chloride and L-cysteine in various ratios, for solutions containing mercuric chloride and EDTA in various ratios and for biological fluids such as blood, serum, and urine. All absorption traces using the Pt loop were obtained with the platinum loop inside the atomizer as shown in Figure 48. Placing the loop outside the atomizer did not work because volatilized species merely condensed on the glass sleeve and were not pulled into the atomizer.

1. Aqueous Solutions of Mercury Compounds

Resonance absorption traces of water, the pure mercury compounds and their molecular background traces are shown in Figures 52-61. In all cases, the traces from the glass rod were broader than those from the Pt loop, due to the slower rate of heating.

2. Mercury-EDTA Complex Solutions

Solutions containing various ratios of $HgCl_2$ and EDTA were studied using the glass rod. The EDTA-Hg system was of interest because whole blood samples which were obtained from the LSU Student Health Service were preserved with EDTA. Four different solutions were studied:

a. 0.05 mM $HgCl_2$

b. 0.05 mM $HgCl_2$ and 0.01 mM EDTA

c. 0.02 mM $HgCl_2$ and 0.03 mM EDTA

d. 0.03 mM EDTA

All solutions were acidified to pH 3 with nitric acid. Absorption traces are shown in Figure 62. A quantitative study of

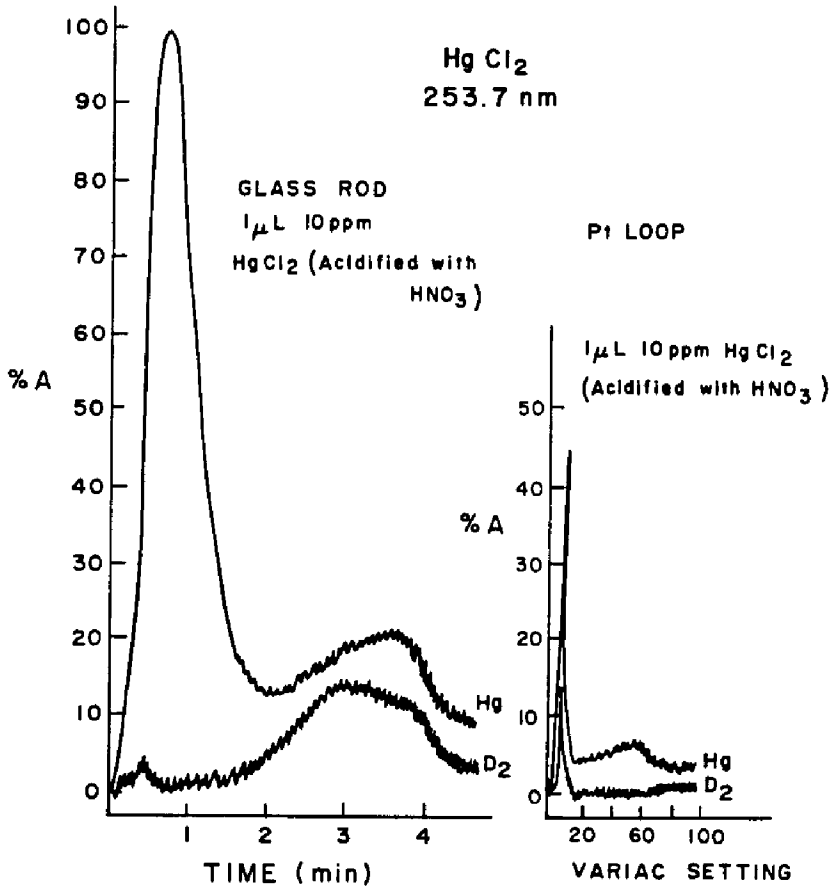


FIGURE 52: ABSORPTION TRACES OF AQUEOUS $HgCl_2$ ON THE GLASS ROD AND Pt LOOP. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION ARE SHOWN.

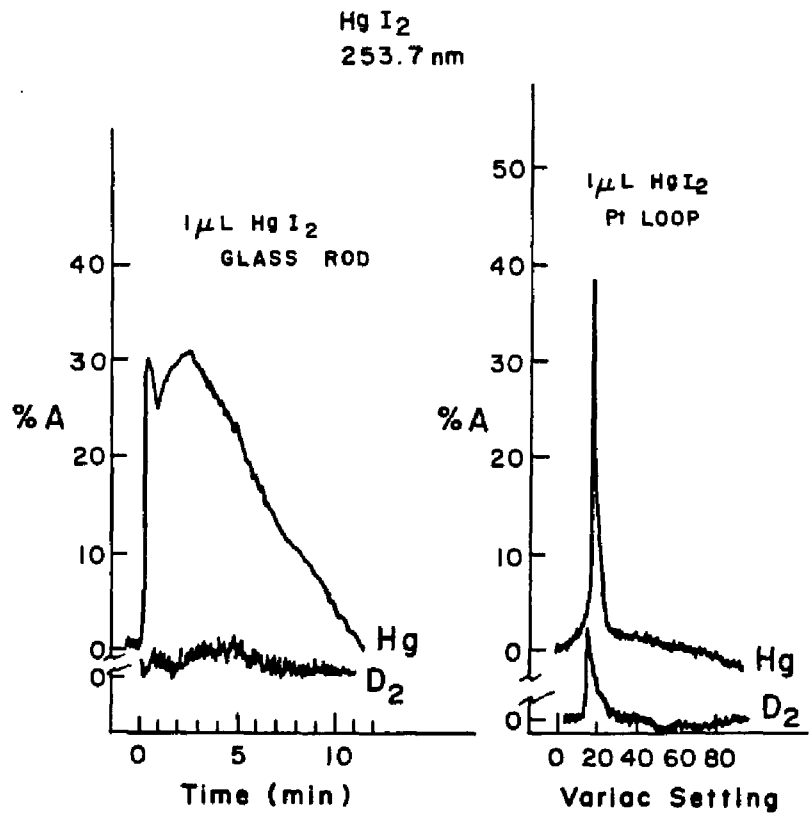


FIGURE 53: ABSORPTION TRACES OF AQUEOUS HgI_2 ON THE GLASS ROD AND Pt LOOP. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION ARE SHOWN.

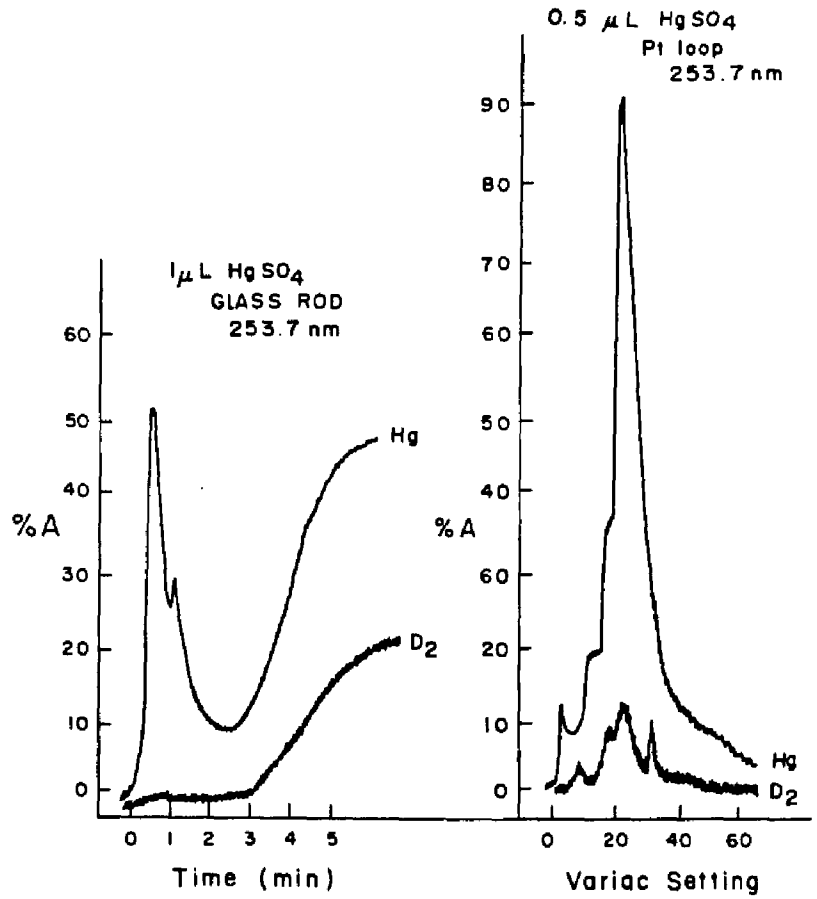


FIGURE 54: ABSORPTION TRACES OF AQUEOUS $HgSO_4$ ON THE GLASS ROD AND Pt LOOP. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION ARE SHOWN.

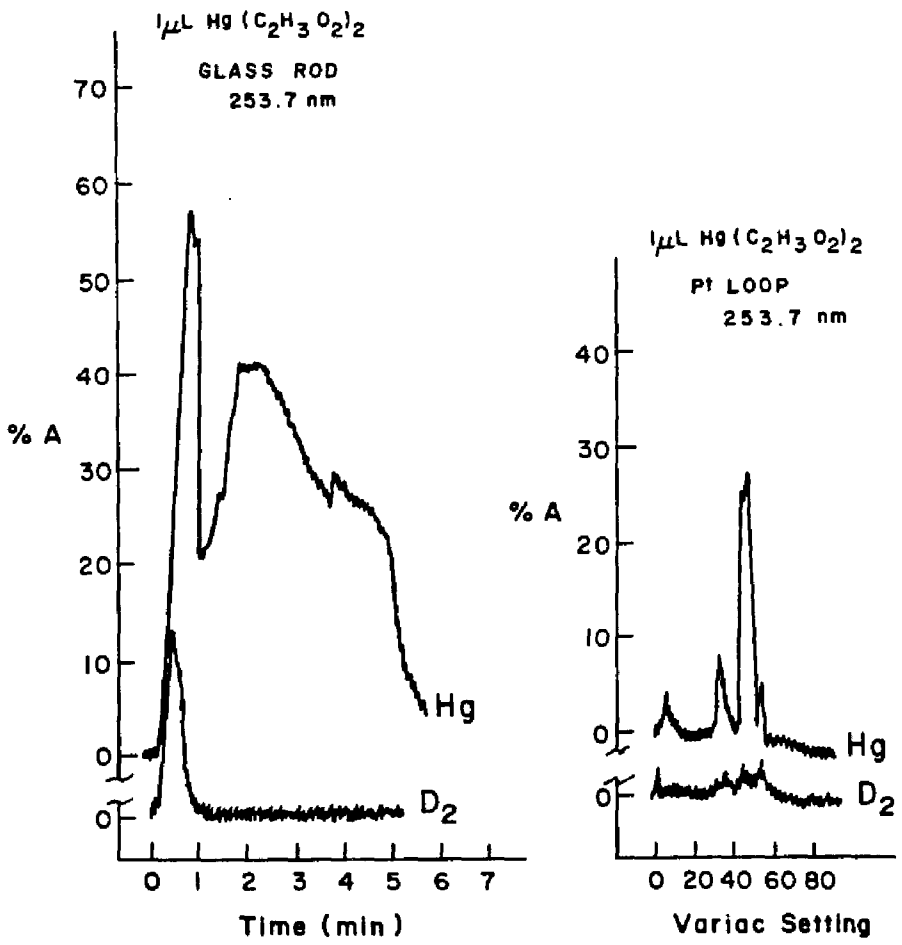


FIGURE 55: ABSORPTION TRACES OF AQUEOUS MERCURIC ACETATE ON THE GLASS ROD AND Pt LOOP. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION ARE SHOWN.

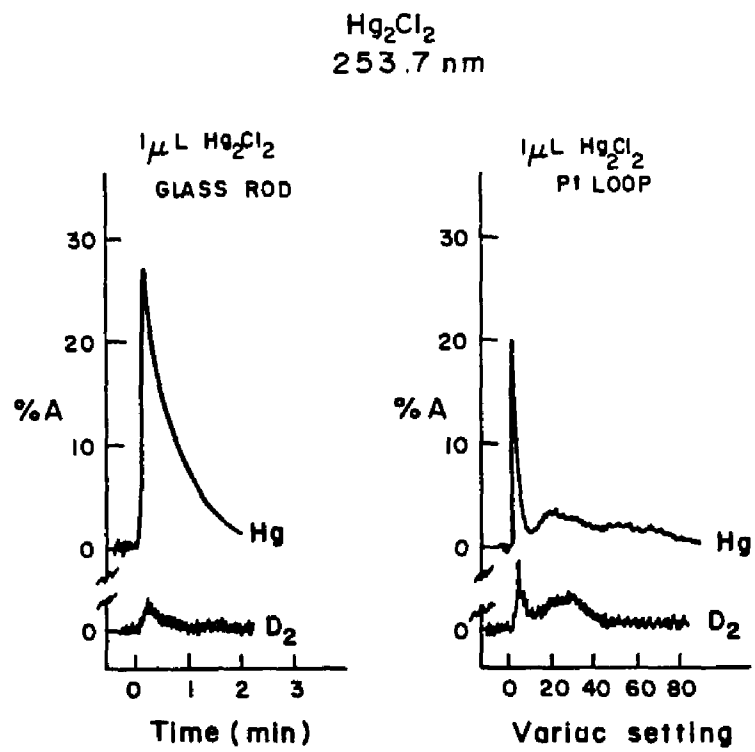


FIGURE 56: ABSORPTION TRACES OF Hg_2Cl_2 ON THE GLASS ROD AND Pt LOOP. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION ARE SHOWN.

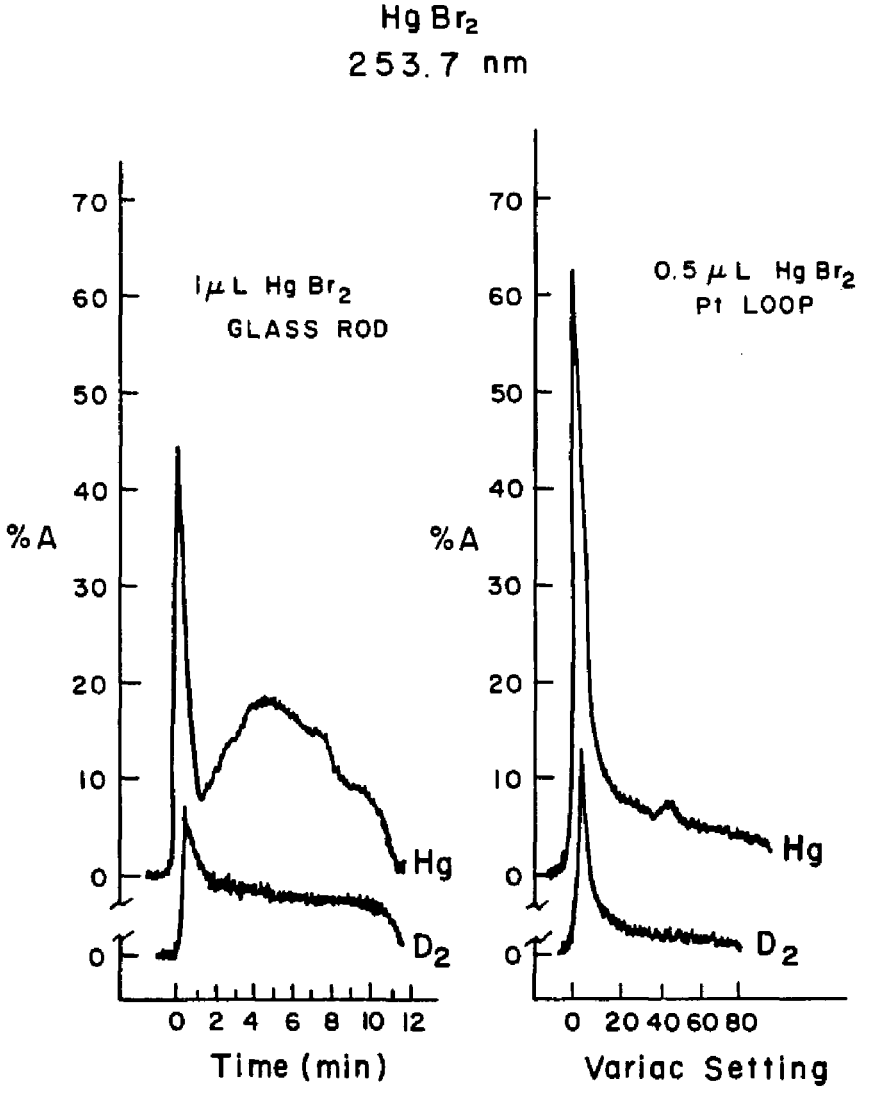


FIGURE 57: ABSORPTION TRACES OF AQUEOUS $HgBr_2$ ON THE GLASS ROD AND Pt LOOP. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION ARE SHOWN.

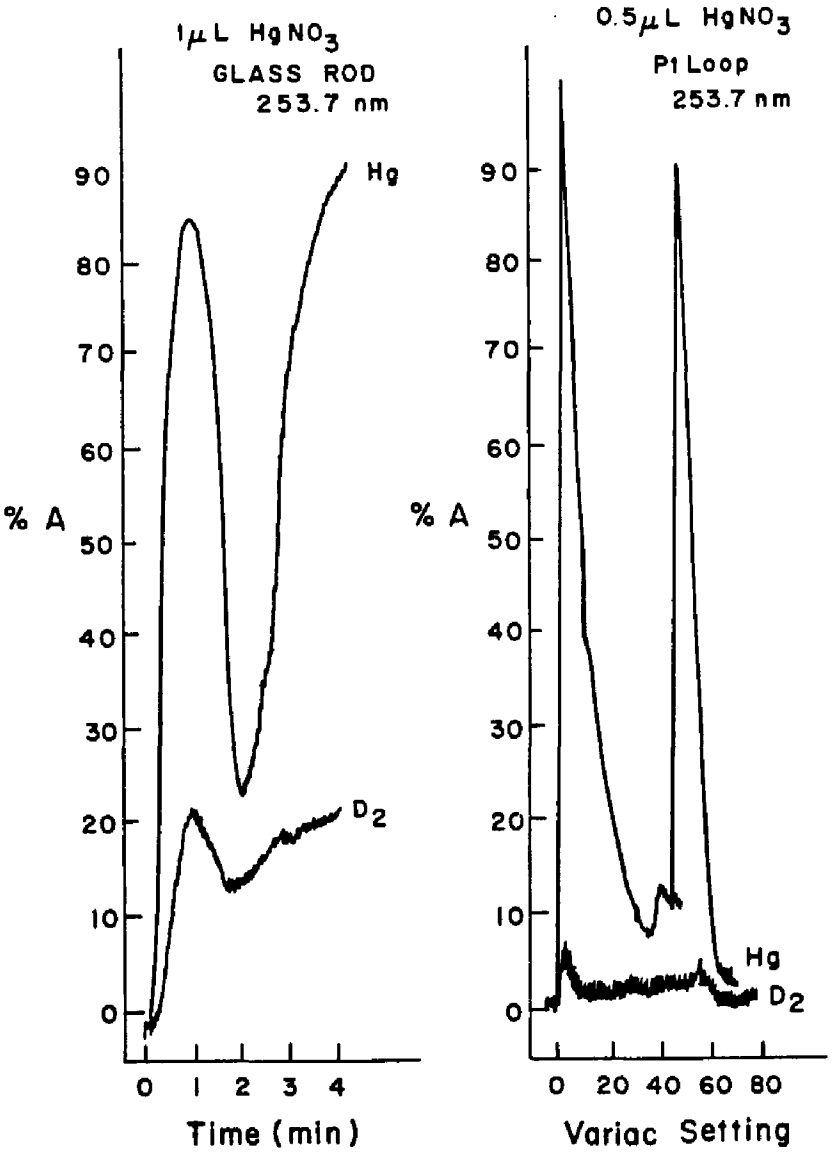


FIGURE 58: ABSORPTION TRACES OF AQUEOUS $HgNO_3$ ON THE GLASS ROD AND Pt LOOP. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION ARE SHOWN.

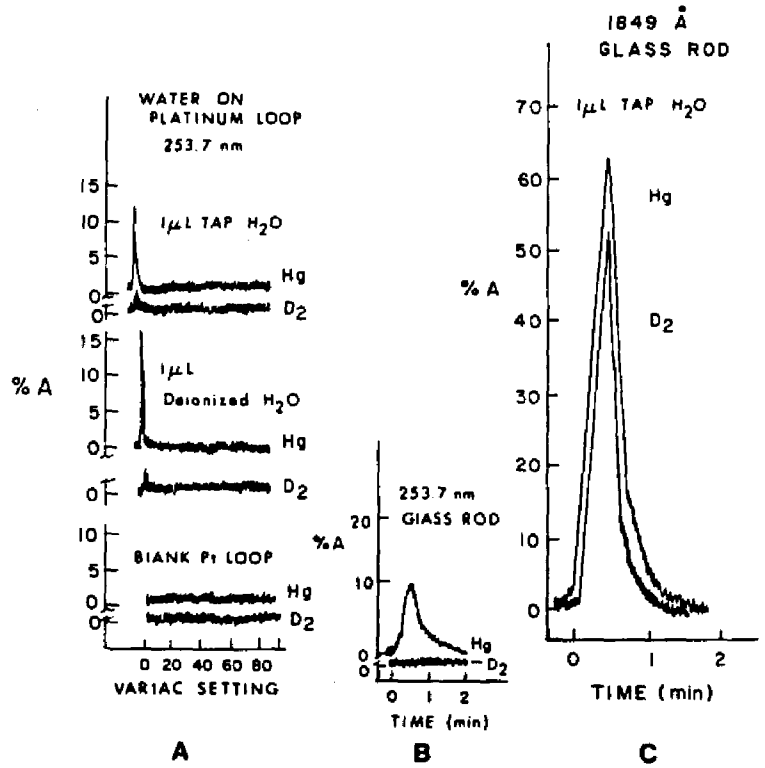


FIGURE 59: ABSORPTION TRACES OF WATER. A: RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION AT 253.7 nm OF TAP AND DEIONIZED WATER ON THE Pt LOOP. B: RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION AT 253.7 nm OF TAP WATER ON THE GLASS ROD. C: RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION AT 184.9 nm OF TAP WATER ON THE GLASS ROD.

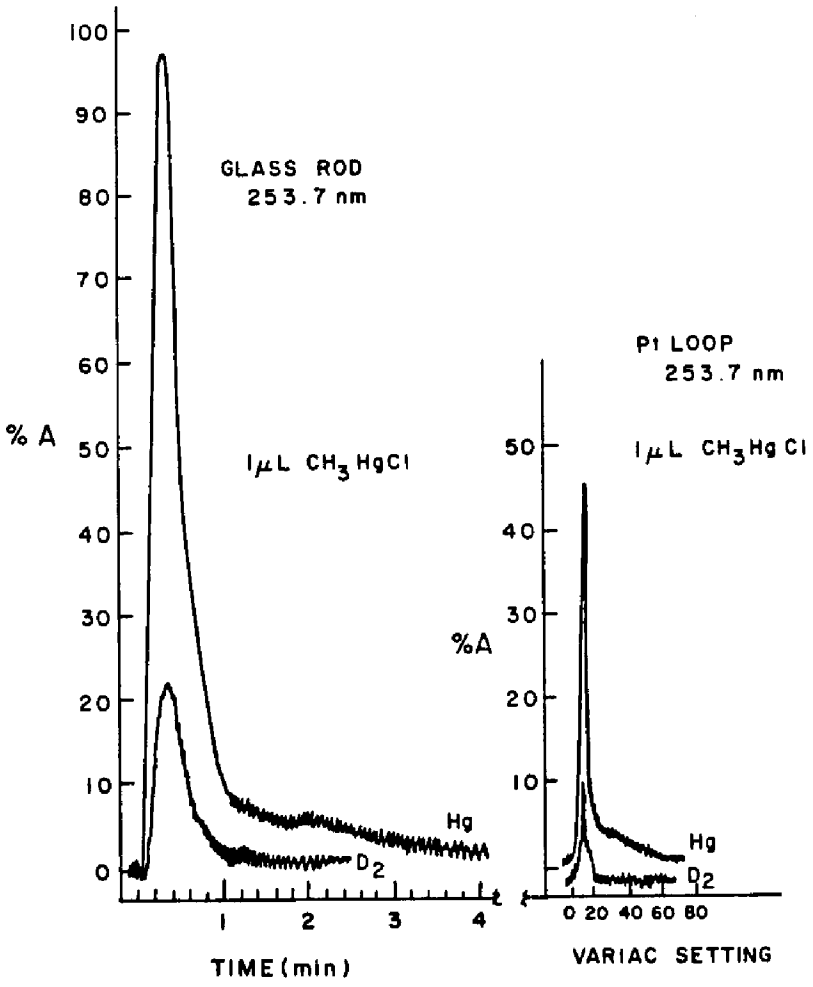


FIGURE 60: ABSORPTION TRACES OF AQUEOUS CH_3HgCl ON THE GLASS ROD AND Pt LOOP. RESONANCE (Hg) AND BACKGROUND(D_2) ABSORPTION ARE SHOWN.

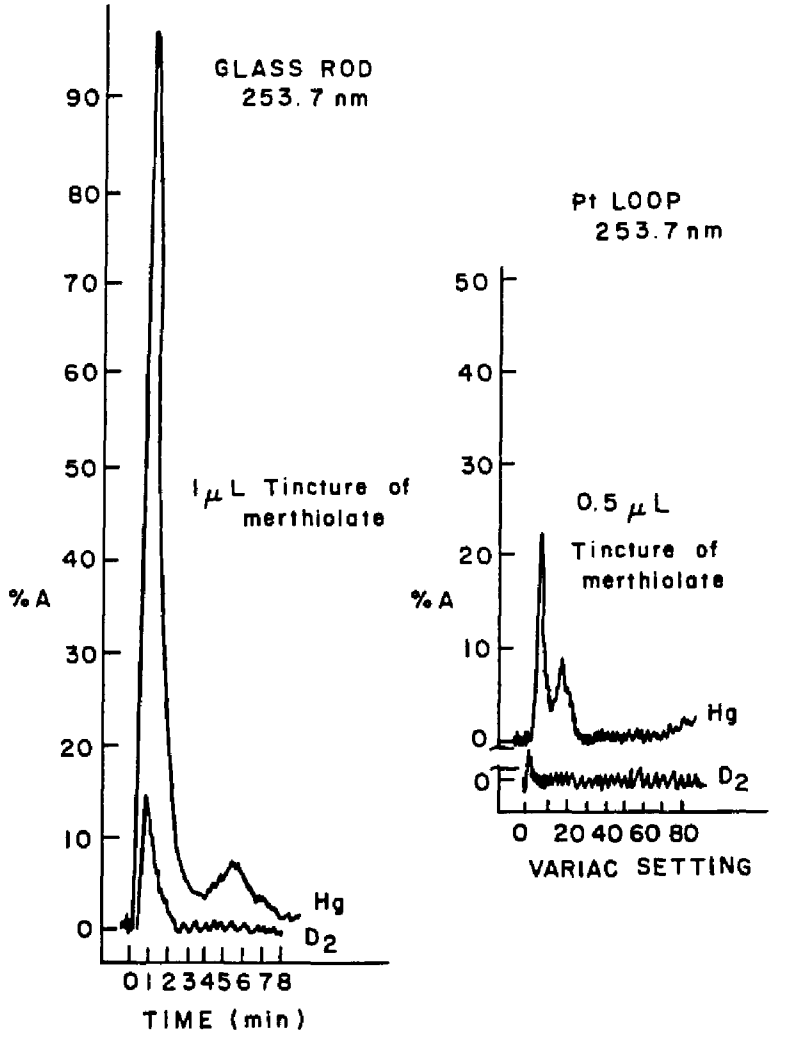


FIGURE 61: ABSORPTION TRACES OF COMMERCIAL "TINCTURE OF MERTHIOLATE" ON THE GLASS ROD AND Pt LOOP. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION ARE SHOWN.

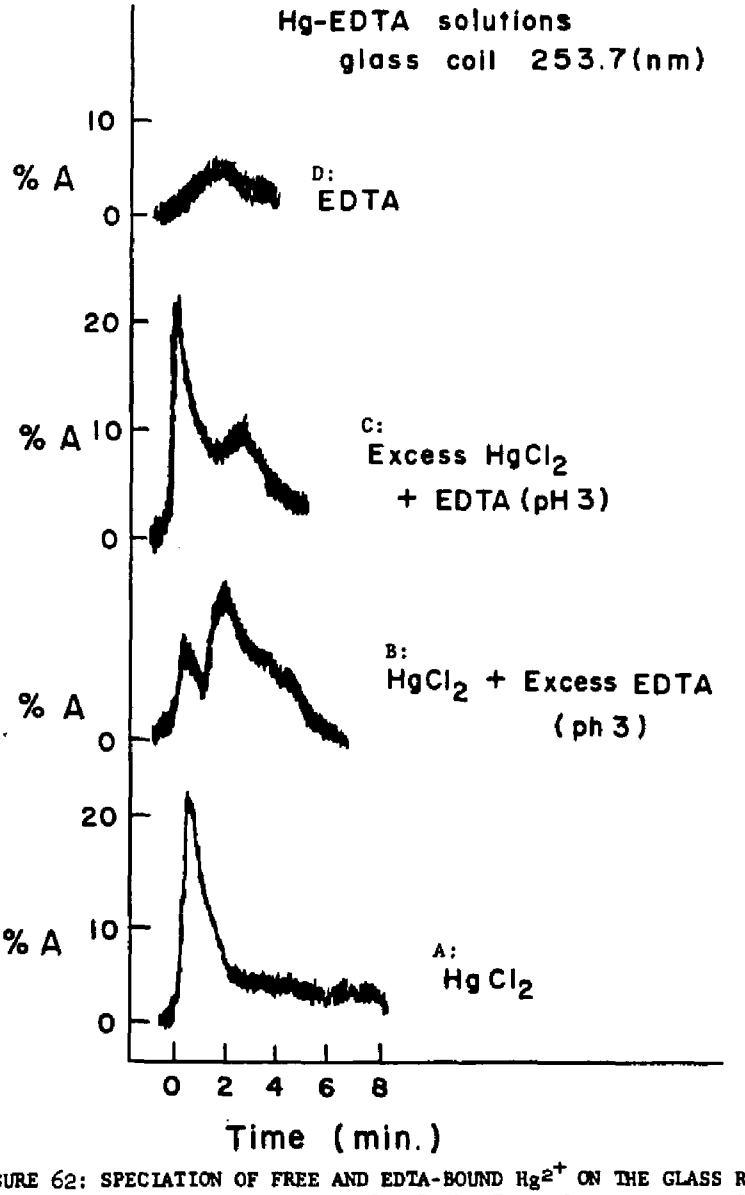


FIGURE 62: SPECIATION OF FREE AND EDTA-BOUND Hg^{2+} ON THE GLASS ROD AT 253.7 nm. A: PURE $HgCl_2$ SHOWED ONE PEAK AT ca. 1 min. B: $HgCl_2$ PLUS EXCESS EDTA SHOWED TWO PEAKS, ONE CORRESPONDING TO $HgCl_2$ AND A LESS-VOLATILE PEAK AT ca. 2 min. C: EXCESS $HgCl_2$ PLUS EDTA SHOWED AN INVERTED INTENSITY RELATIONSHIP BETWEEN THE $HgCl_2$ PEAK AND THE SECOND PEAK COMPARED TO THE PREVIOUS TRACE. D: PURE EDTA SHOWED A SMALL BROAD ABSORPTION PEAK AT ca. 2-3 min.

the EDTA-Hg solutions was made by assuming 100% efficiency of mercury binding to EDTA in the solution and measuring the peak heights. A calibration curve was established by injecting known volumes of Hg vapor-saturated air. From the absorption traces, it appeared that mercuric chloride was more volatile than the Hg-EDTA complex. The quantitative recovery data (Table 35) indicated that the Hg-EDTA complex did not completely evaporate from the glass rod.

3. Mercury-Cysteine Complex Solutions

Solutions containing various ratios of mercuric chloride and L-cysteine were studied using the glass rod. L-cysteine was used as a model for protein-bound mercury. Absorption traces are shown in Figure 63. Two separate mercury-containing peaks were seen in the mercury-cysteine mixtures, which indicated that protein-bound mercury was separated from ionic mercury by this technique.

Subsequently, solutions of mercuric chloride and other organic complexing agents, namely, dithioxamide and dithizone, were studied. The mercury-dithioxamide traces are shown in Figure 64. The mercury-dithizone complex was not water soluble, but the organic solvents in which it was soluble ($CHCl_3$, CCl_4) gave 100% molecular absorption of the 253.7 nm line. Therefore this complex could not be studied.

4. Biological Fluids

Urine, whole blood, serum, saliva and sweat were

Table 35

Mercury-EDTA Complex: Quantitative Studies
Using the Glass Rod Vaporization Stage

Solution	ng Hg^{2+} added	ng unbound Hg^{2+} expected	ng total Hg found
1	10	10	9.3
2	10	8	8.5
3	4	0	2.9
4	0	0	0

(a) as $HgCl_2$ acidified with HNO_3.

(b) assuming 100% efficiency of Hg-EDTA complex formation.

(c) corrected for deionized distilled water blank value of 0.3 ng Hg/μL

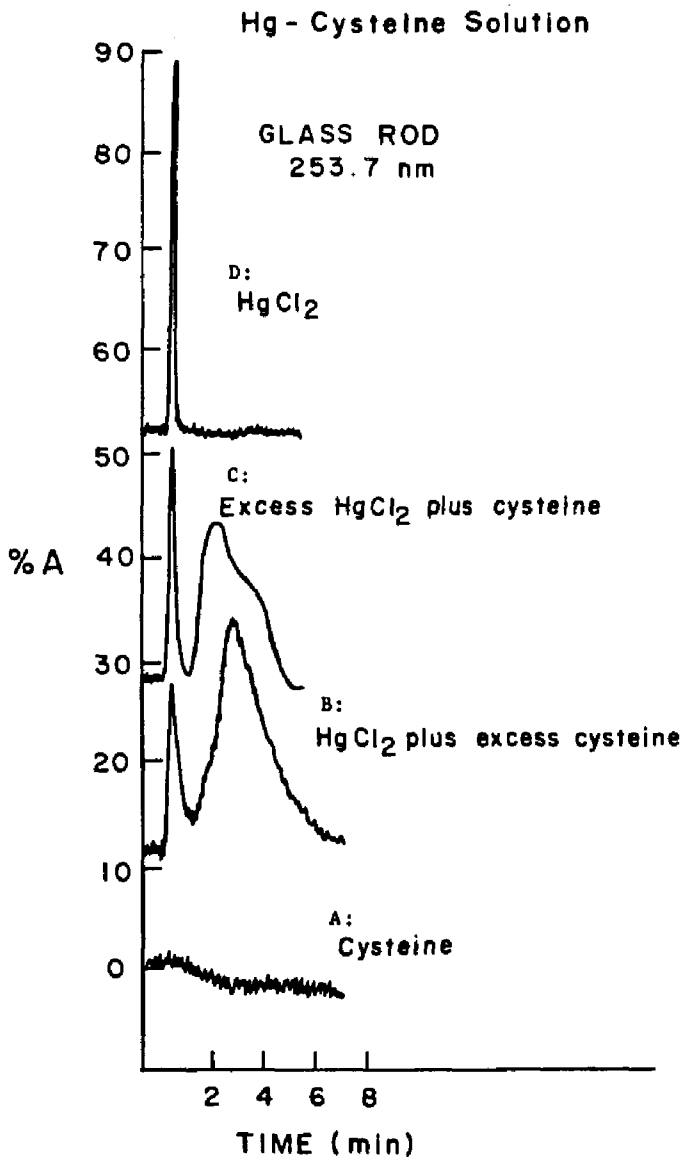


FIGURE 63: SPECIATION OF FREE AND CYSTEINE-BOUND Hg^{2+} ON THE GLASS ROD AT 253.7 nm. A: AQUEOUS CYSTEINE SOLUTION GAVE NO ABSORPTION. B: $HgCl_2$ PLUS EXCESS CYSTEINE SHOWED TWO PEAKS, CORRESPONDING TO $HgCl_2$ AND A LESS VOLATILE MERCURY COMPOUND. C: EXCESS $HgCl_2$ PLUS CYSTEINE SHOWED AN INVERTED INTENSITY RELATIONSHIP BETWEEN THE TWO PEAKS COMPARED TO THE PREVIOUS TRACE. D: PURE $HgCl_2$ SHOWED ONE ABSORPTION PEAK AT ca. 1 min.

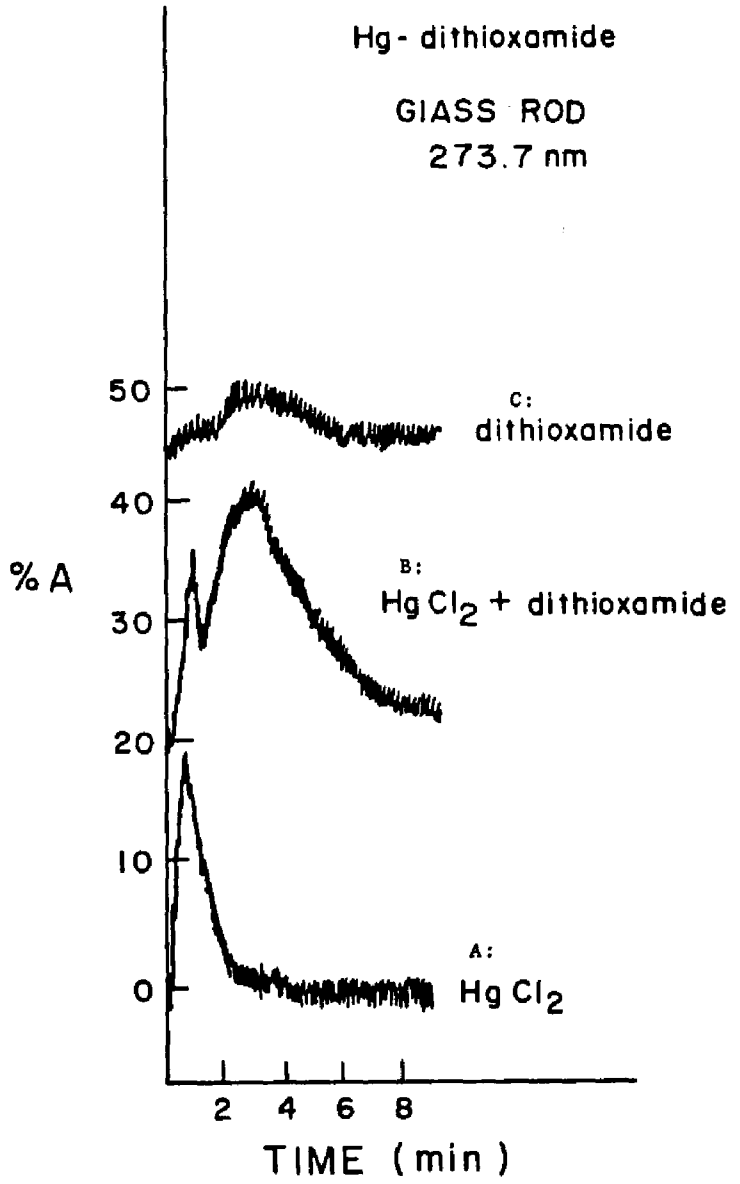


FIGURE 64: SPECIATION OF FREE AND DITHIOXAMIDE-BOUND Hg^{2+} ON THE GLASS ROD AT 253.7 nm. A: AQUEOUS $HgCl_2$ SHOWED ONE PEAK AT ca. 1 min. B: $HgCl_2$ PLUS DITHIOXAMIDE SHOWED TWO PEAKS, ONE CORRESPONDING TO $HgCl_2$ AND ANOTHER LESS VOLATILE MERCURY COMPOUND PRESUMED TO BE MERCURY BOUND TO DITHIOXAMIDE. C: AQUEOUS DITHIOXAMIDE ALONE SHOWED ONLY A SMALL, BROAD PEAK AT ca. 3 min.

analyzed using both the platinum loop and glass rod. Absorption traces are given in Figures 65-70. A single absorption peak was obtained from urine with about the same appearance time as the mercuric and methylmercuric halides. Whole blood (EDTA preserved) gave multiple peaks at 184.9 nm, but a barely discernible trace at 253.7 nm due to the low level of mercury present. No absorption was seen for serum at 253.7 nm. Saliva gave a small broad peak with an appearance time equal to that for urine. Sweat gave a small broad peak with an appearance time later than that of the mercuric halides but less than that for cysteine-bound mercury. An artificial sweat sample was made from NaCl and KCl to approximate the sweat matrix. This saline solution was spiked with mercuric chloride, but the appearance time of mercuric chloride was not changed.

A summary of absorption maxima found for each compound under the various conditions used is given in Table 36.

An attempt was made to analyze segments of hair by placing segments within the loop or coil. The attempt was not successful for several reasons. Small hair segments (1 cm length) were pulled or blown off the loop by the gas flow through the system. Longer hair segments tended to hit the side of the atomizer and started to burn. The hair segments which remained in place on the glass coil were incompletely volatilized and tended to char on the glass. No conclusive data on speciation in solid hair samples was obtained.

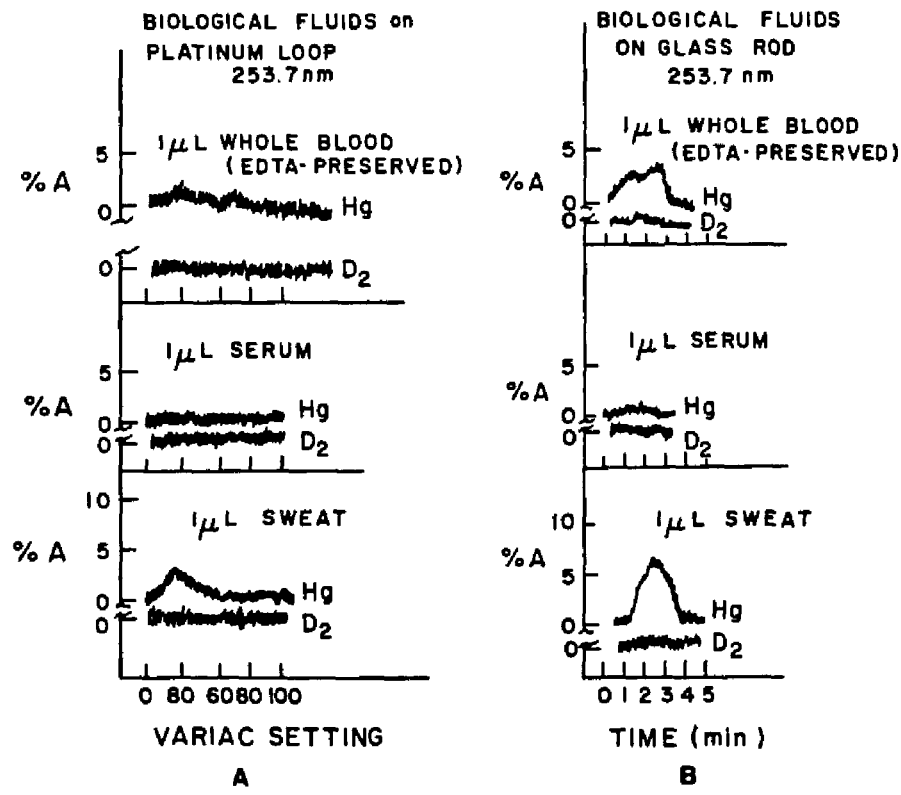


FIGURE 65: ABSORPTION SIGNALS OF WHOLE BLOOD, SERUM AND SWEAT AT 253.7nm ON THE Pt LOOP (A) AND THE GLASS ROD (B). RESONANCE (Hg) AND BACKGROUND ABSORPTION (D_2) SIGNALS ARE SHOWN.

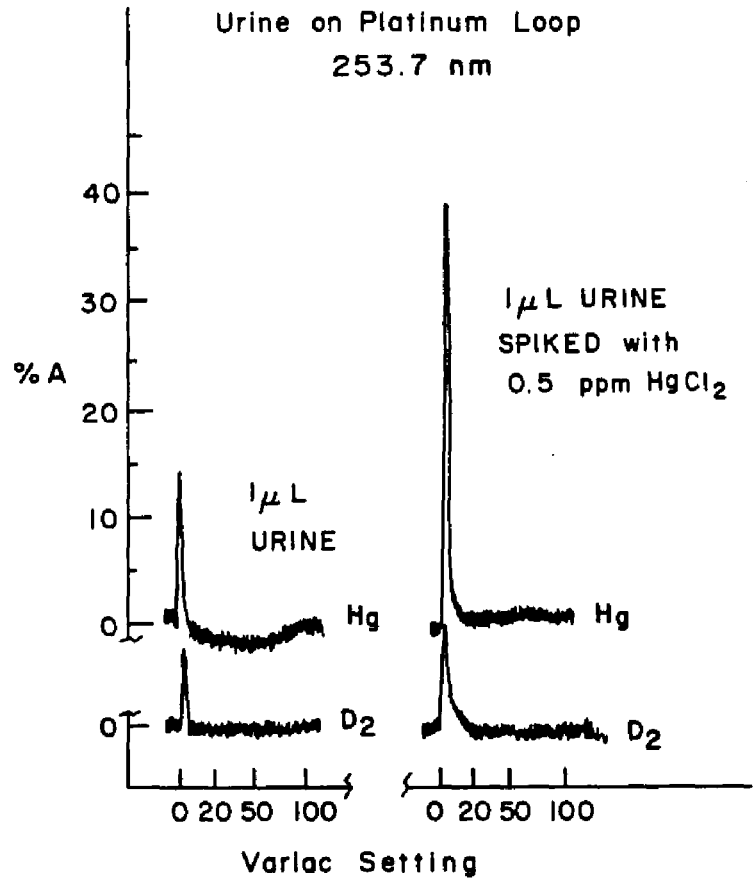


FIGURE 66: ABSORPTION SIGNALS FOR URINE AND URINE SPIKED WITH $HgCl_2$ AT 253.7 nm ON THE Pt LOOP. NO DIFFERENCE IN APPEARANCE TIME BETWEEN THE SIGNALS FROM SPIKED AND UNSPIKED URINE WAS SEEN. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION ARE SHOWN.

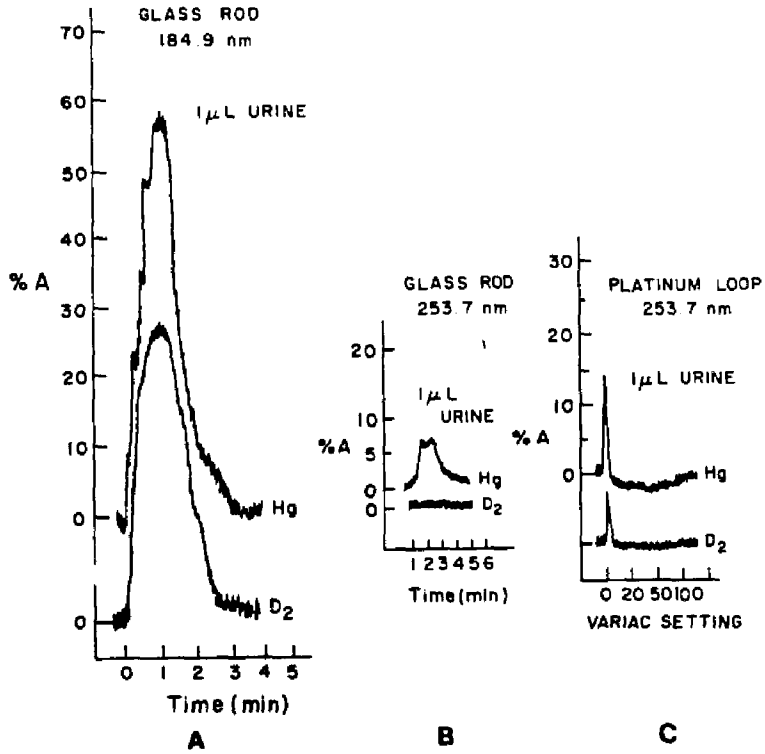


FIGURE 67: ABSORPTION TRACES FOR URINE ON THE GLASS ROD AND Pt LOOP. A: ABSORPTION AT 184.9 nm BY URINE ON THE GLASS ROD B: ABSORPTION AT 253.7nm BY URINE ON THE GLASS ROD. C: ABSORPTION AT 253.7 nm BY URINE ON THE Pt LOOP. RESONANCE (Hg) AND BACKGROUND (D_2) ABSORPTION ARE SHOWN.

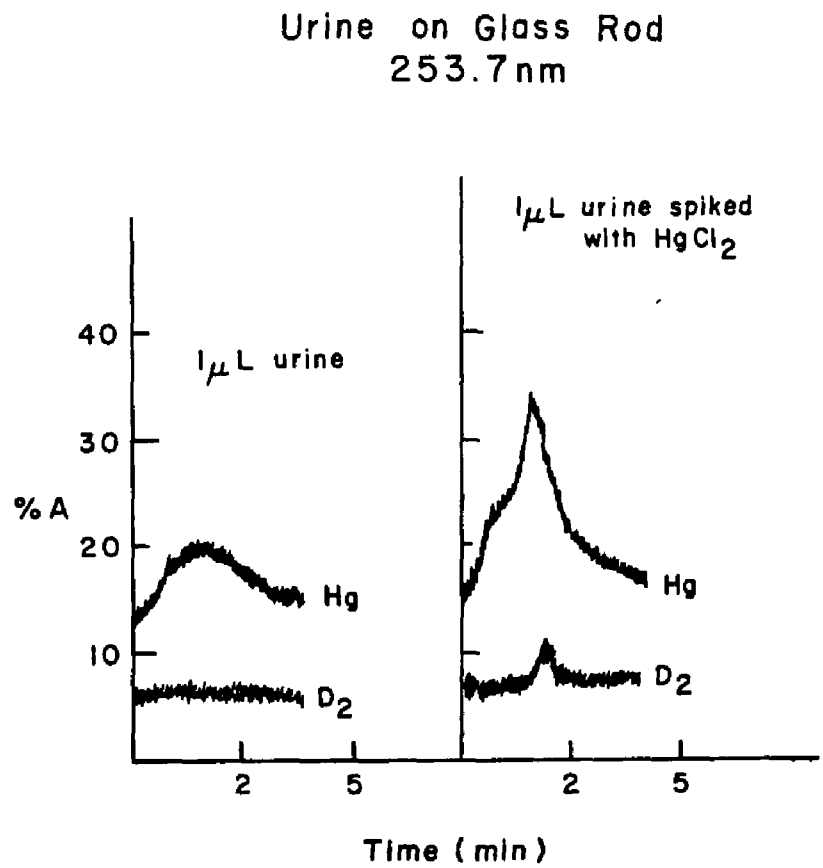


FIGURE 68: ABSORPTION TRACES OF URINE AND URINE SPIKED WITH $HgCl_2$ ON THE GLASS ROD AT 253.7 nm. IN CONTRAST TO THESE SAME SAMPLES ON THE Pt LOOP (FIG.66), THE SPIKED SAMPLE EXHIBITED TWO PEAKS, A SHARP ONE SUPERIMPOSED UPON A BROAD PEAK. THIS DISCRIMINATION IS DUE TO THE SLOWER HEATING RATE OF THE GLASS ROD COMPARED TO THE Pt LOOP.

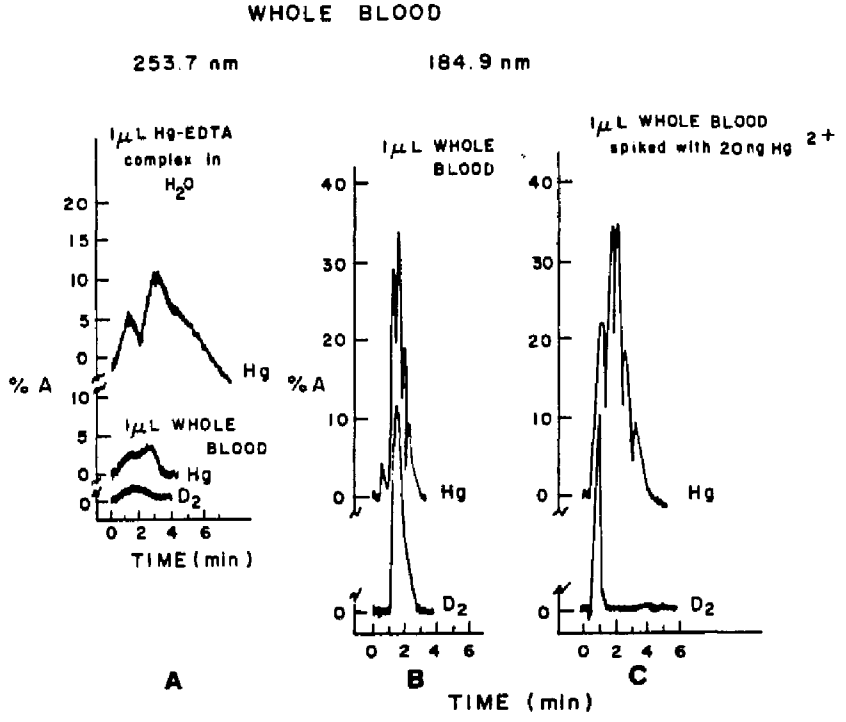


FIGURE 69: ABSORPTION TRACES OF WHOLE BLOOD (EDTA-PRESERVED) ON THE GLASS ROD. A: ABSORPTION SIGNALS FROM EDTA-PRESERVED WHOLE BLOOD AND AQUEOUS Hg-EDTA SOLUTION AT 253.7 nm. TWO PEAKS ARE SEEN, WHICH MAY BE DUE TO FREE AND BOUND Hg. B: WHOLE BLOOD ON THE GLASS ROD AT 184.9 nm. MULTIPLE PEAKS ARE PROBABLY DUE TO UNEVEN HEATING OF THE SAMPLE, SINCE SPIKING WITH $HgCl_2$ AS IN (C) INCREASED THE ENTIRE AREA UNDER THE PEAKS.

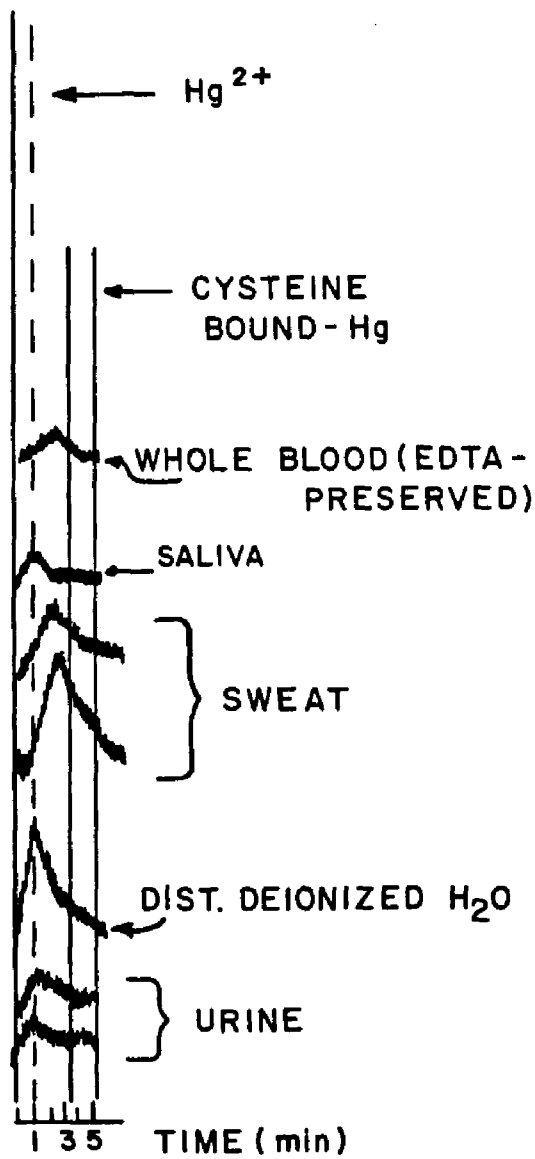


FIGURE 70: APPEARANCE TIMES OF MERCURY-CONTAINING PEAKS IN VARIOUS SAMPLES, COMPARED TO $HgCl_2$ (DOTTED LINE) AND CYSTEINE-BOUND MERCURY (WITHIN SOLID LINES).

Table 36

Summary of Mercury Speciation Data

Compound or Sample	Boiling Point, °C	Conditions for Attaining Maximum Absorption at 253.7 nm: Platinum Loop: Variac Setting	Glass Rod: Elapsed Time in Bed (min)
Hg_2Cl_2	400 (s)	5, 50	0.5
$HgCl_2$	276	5	0.5
$HgBr_2$	322	5, 40	0.5, 5, 7.5
HgI_2	354	10	0.5, 3
$HgSO_4$	d	5, 25, 45, 65	0.8, 1.5, 4
$Hg(C_2H_3O_2)_2$	d	35, 45, 50	0.5, 2.5, 3.8, 4.5
$Hg_2(NO_3)_2$	d	5, 55	1.0, 4
CH_3HgCl	100	5	0.5
$Hg(C_6H_5)_2$	204	5	0.5
p-OH-phenyl HgCl	260	5	0.5
Tap water	100	5	0.5
Urine		5	1.0
Saliva			1.0
Sweat		15	1.5
Whole blood			1.0
Serum		Not detected	Not detected
Tincture of Merthiolate			1.0, 5.5
$HgCl_2$-cysteine			0.5, 3.5
$HgCl_2$-EDTA			0.5, 3

(a) Data from CRC Handbook of Chemistry and Physics, 56th Ed., CRC Press: Cleveland, Ohio, 1975.

(b) EDTA treated.

Note: Because of the difficulty in estimating the temperature of the platinum loop and the glass rod when the absorption maximum was reached, the data are expressed in time or variac setting for comparison purposes only.

D. DISCUSSION

1. Temperature Within the System

From an analysis of the absorption traces, it appeared that speciation of some mercury compounds could be performed by volatilization from either the platinum loop or glass rod. Mercury bound to an organic sulfhydryl group could be separated from mercury bound to a halide, for example. The mercuric sulfate, mercurous nitrate and mercuric acetate salts gave characteristic peaks corresponding to volatilized species. Unfortunately the temperature control of the system was not adequate to separate the majority of mercury salts due to their high volatilities.

The temperature control within the system left much to be desired. The atomizer carbon bed had to be maintained at 1500°C in order to effect atomization of volatilized species, but this resulted in a temperature of about 250°C at the position of the glass coil and platinum loop. Thus the platinum loop was rapidly heated even with no application of voltage. The glass coil heated a bit more slowly, as evidenced by the broader absorption traces, but still appeared to reach 250° very rapidly. This rapid heating was undoubtedly sufficient to vaporize immediately water and most of the simple mercury salts and organomercury compounds examined. It was impossible to determine the temperature of the platinum loop as a function of the applied voltage using an optical pyrometer, since all of the absorption peaks appeared at Variac

settings well below those at which the wire became incandescent. The optical pyrometer was used to measure the temperature of the loop at high Variac settings and a possible extrapolation to lower temperatures is given in Figure 71, but an exact correspondence between loop temperature and Variac setting could not be obtained. It was likewise impossible to measure the temperature of the glass coil, so the appearance time of the absorption peak was measured, with the assumption that the convective heating of the coil was reproducible from sample to sample.

It had been hoped that placing the Pt loop outside the atomizer would allow finer temperature control between 25°-250°C. Unfortunately, species which volatilized from the wire condensed on the wall of the glass sleeve, which was at room temperature, instead of being carried into the atomizer.

2. Qualitative and Quantitative Analysis

The main objective of this study was to determine qualitatively the speciation of mercury compounds but the dual stage system could be used for quantitative analysis. Pipetting known volumes of samples and standards onto the loop or coil allowed preparation of a calibration curve and ready quantitation of mercury concentrations. Mercury levels in water determined by volatilization from the platinum loop and glass coil compared favorably with concentrations determined by direct injection of water samples onto the carbon bed. Peaks from the platinum loop were much sharper than those from the glass coil. Quantitation

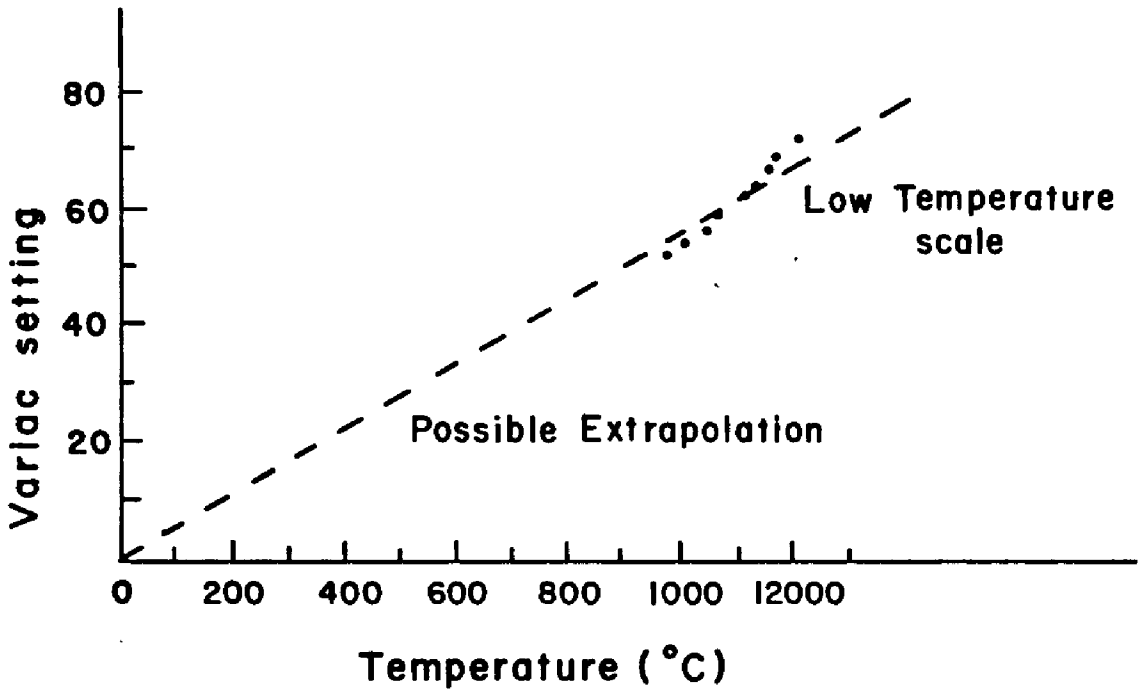


FIGURE 71: PLOT OF TEMPERATURE OF THE Pt LOOP AS A FUNCTION OF VARIAC SETTING, AS DETERMINED WITH AN OPTICAL PYROMETER. DOTS REPRESENT POINTS MEASURED WITH THE PYROMETER SET ON ITS LOW TEMPERATURE SCALE. TEMPERATURES BELOW 900°C COULD NOT BE MEASURED WITH THE PYROMETER, BUT THE DASHED LINE REPRESENTS A POSSIBLE EXTRAPOLATION.

was easier on the platinum loop, since peak heights could be measured, whereas peak area had to be measured for the broad peaks from the glass coil. The absorption traces from the glass coil were more reproducible than those from the platinum loop. The loop became soft upon repeated heating cycles and appeared to heat unevenly with prolonged use, causing irreproducible volatilization.

The 184.9 nm line was significantly more sensitive than the 253.7 nm line, but molecular background from H_2, CO and other decomposition products was also significantly greater. The signals generated by 1 μL of tap water and 1 μL of urine volatilized from the glass coil were measured at both wavelengths and are compared in Figures 59 and 67. The glass coil could be used to study volatilization of mercury compounds at 184.9 nm by inserting it through the brass cap designed to allow N_2 purging of the light path.[35] The platinum loop had to be fitted directly into the atomizer head and could not be used at 184.9 nm. Removal of the loop in order to inject a sample allowed air into the atomizer, which caused complete absorption of the 184.9 nm line. The mercury compounds all volatilized before 100% transmission of the light source was reattained.

3. Solutions of Pure Mercury Compounds

Almost all of the solutions investigated gave an immediate peak upon introduction of the loop or the glass coil into the atomizer. For the inorganic chlorides, organomercury compounds

and water, this was the only peak seen. This was not surprising since the temperature at the atomizer inlet was greater than or equal to the boiling points of these compounds. In the cases of the higher boiling salts, it was not inconceivable that this initial peak was due to mercury compounds entrained in the rapidly evaporated solvent, but was more likely indicative of some dissociation of the salt to give Hg^{2+} ion. For the $HgSO_4$ and $Hg(C_2H_3O_2)_2$ solutions, this initial peak was more prominent on the glass coil than on the platinum loop. The reason for this was not understood.

Diphenylmercury and p-hydroxyphenyl mercuric chloride gave absorption traces on the glass coil identical to that of methylmercuric chloride and are not shown.

Most of the inorganic mercury compounds were molecular rather than ionic compounds. There appeared to be a certain amount of dissociation of all the mercuric halides however, even in neutral solution. For example, Figure 72 showed the effect of dilution on the absorption trace of saturated $HgBr_2$ solution. The saturated supernatant showed 2 peaks, one sharp peak which emerged immediately and a large broad peak centered at 3.5 minutes. On dilution, the first peak did not change significantly, but the second peak was greatly reduced. Further dilution did not change the first peak, but almost completely eliminated the second peak. It seemed logical to assign the first peak to ionic Hg^{2+} and the second to molecular $HgBr_2$ but this was unsubstantiated.

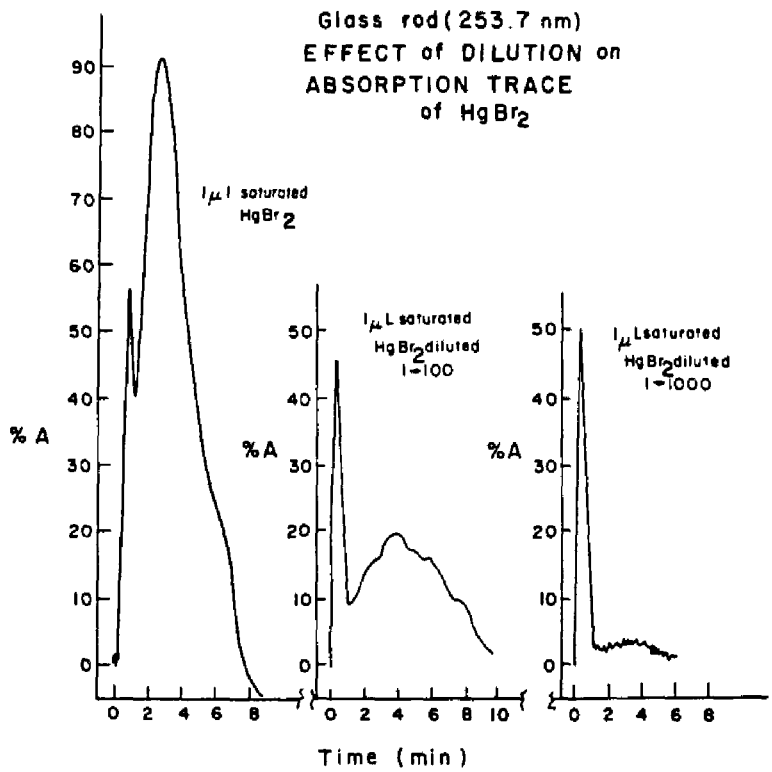


FIGURE 72: ON DILUTION OF A SATURATED AQUEOUS SOLUTION OF $HgBr_2$, THE SECOND PEAK DECREASED IN ABSORPTION INTENSITY. THE SECOND PEAK COULD BE DUE TO UNDISSOCIATED $HgBr_2$. THE FIRST PEAK CORRESPONDED TO Hg^{2+}.

4. Hg-EDTA Studies

EDTA was added to solutions of $HgCl_2$ to observe the effect of this complexing agent on the absorption traces. The pH of the solutions was adjusted to 3-4 to stabilize the Hg-EDTA complex. Solutions (previously described) of $HgCl_2$ only, EDTA only, excess $HgCl_2$ plus EDTA and $HgCl_2$ plus excess EDTA were studied. As can be seen in Figure 62, one peak was obtained for $HgCl_2$ alone while two peaks were obtained in the HgCl-EDTA mixtures. The first of the two peaks had an appearance time equal to that of pure $HgCl_2$. The height of the first peak decreased on changing from excess $HgCl_2$ to excess EDTA, while the height of the second peak increased. It seemed reasonable to assume that the first peak represented Hg^{2+} and the second peak, the Hg-EDTA complex. Studies (Table 35) indicated that the Hg-EDTA complex was not quantitatively volatilized from the glass rod. This was confirmed by the appearance of charred organic material on the glass coil when the coil was held in a Bunsen burner flame for cleaning.

5. Hg-Cysteine Studies

It was well-known that very strong binding of mercury to organic sulfhydryl groups occurred.[196] Mercury was thought to be bound to sulfur-containing amino acids throughout the body. Solutions containing mercuric chloride and L-cysteine were prepared to simulate mercury-protein systems in the body. The absorption traces are shown in Figure 63. Cysteine itself (10 mM

aqueous solution) gave no absorption peaks. Mercuric chloride gave its typical peak at about 0.5 minutes after injection. Mixtures of $HgCl_2$ and cysteine gave two distinct peaks, one corresponding to the $HgCl_2$ peak and a broad peak centered at about 3.5 minutes. The second peak increased with increasing cysteine/Hg ratio and can probably be attributed to a mercury-cysteine complex. This indicated that separation of ionic and protein-bound mercury could be performed by this simple, rapid technique with no prior sample treatment.

Solutions of mercuric chloride with dithioxamide (rubeanic acid), another sulfhydryl-containing organic, were studied using the glass coil. Results similar to those of the Hg-cysteine solutions were obtained. Two peaks were seen in the Hg-dithioxamide mixture, one corresponding to $HgCl_2$ and a second peak centered at 3.5 minutes. A shoulder appeared at about 5 minutes. Dithioxamide itself gave 2 small, broad peaks at about 1 and 4 minutes.

6. Biological Fluids

a. Urine

Urine samples on the platinum loop gave a sharp peak corresponding to that obtained for both $HgCl_2$ and CH_3HgCl. Urine spiked with $HgCl_2$ gave the same single sharp peak (Figure 66). In contrast, urine samples on the glass coil gave a broad peak (from 0.5-2 minutes) which could be attributed to $HgCl_2$. However, urine samples spiked with $HgCl_2$ showed a

distinct peak which appeared between 1 and 2 minutes, later than the appearance time of $HgCl_2$ (Figure 68). The appearance time of $HgCl_2$ was compared in aqueous solution and in saline solution (used to simulate urine) to see if a matrix effect existed, but the appearance time of the two peaks was identical. It was possible that added $HgCl_2$ complexed with protein material in the urine sample, resulting in a delayed appearance time. Not all of the urine volatilized off the glass coil, as evidenced by a whitish salt-like deposit on removing the coil from the atomizer and observation of charring organic material on cleaning the coil in the Bunsen burner.

b. Whole Blood (EDTA-preserved) and Serum

Whole blood on the platinum loop did not give any definitive signal at 253.7 nm. On the glass coil at 253.7 nm, whole blood gave a small (<5% A) absorption signal which appeared to contain at least two peaks (Figures 65 and 69). The absorption trace for aqueous Hg-EDTA solution is also presented in Figure 69 for comparison since the blood was treated with EDTA. At 184.9 nm on the glass coil, whole blood gave a series of peaks. On addition of $HgCl_2$, the entire area under the peaks increased but no single peak appeared to increase dramatically. This indicated that the multiple peaks were probably due to uneven heating of the viscous blood sample and not to separate Hg-containing species. The blood rapidly coagulated on exposure to the heat of the carbon bed and left a spongy charred residue on

the end of the coil.

No absorption signal was observed for serum.

c. Saliva

A small broad peak was seen for saliva samples analyzed at 253.7 nm on the glass rod. The mercury compound had the same appearance time as that in urine and was more volatile than that in sweat.

d. Sweat

Sweat samples gave a small peak with an appearance time later than that of $HgCl_2$ (Figures 65 and 70) but earlier than that for Hg-cysteine. Salt concentrations similar to those found in sweat did not affect the appearance time of $HgCl_2$. Therefore, the mercury compound in sweat was probably not ionic mercury or $HgCl_2$.

The relationship between the absorption traces for biological fluids, Hg^{2+} and Hg-cysteine is most easily seen in Figure 70.

E. CONCLUSIONS AND SUMMARY

1. The dual stage atomization technique described allowed speciation of several mercury-containing compounds in aqueous solution and in biological fluids. The technique held great promise for further speciation studies.

2. Accurate temperature control, especially at temperatures less than 200°C, was needed to separate the extremely volatile mercury halides and simple organomercurials from each other. This

may best be accomplished by redesign of the first stage to place it outside the quartz "T". For example, simultaneous heating of the loop and the glass sleeve should prevent condensation on the walls of the sleeve.

3. Studies with mercury salts and EDTA, L-cysteine and dithioxamide demonstrated that this technique may be used to study the extent of complex formation.

4. Investigations of biological fluids indicated that there was a single predominant form of mercury in sweat, a single predominant form in saliva and a single predominant form in urine. The mercury compound in urine and that in saliva were more volatile than that in sweat.

5. Both quantitative and qualitative analyses were possible with this technique.

GENERAL CONCLUSIONS

In summary, the following general conclusions can be made.

1. Atomic absorption spectroscopy using the quartz "T" atomizer was shown to be a successful technique for the direct determination of mercury in environmental and biological materials. The technique was used in the direct analysis of water, urine, sweat, whole blood, serum, breath, saliva and hair samples. The liquid samples were introduced into the carbon bed on carbon disks or by direct injection with a microdispenser. Hair samples were dropped into the atomizer by tweezers. Breath samples were adsorbed on the carbon bed itself.

Use of the quartz "T" atomizer had several advantages. More complete degradation of the organic matrix of biological samples occurred. This successfully reduced molecular background absorption. The design of the atomizer permitted use of the more sensitive 184.9 nm resonance line as well as the spin-forbidden 253.7 nm line. The technique was very sensitive; as little as 1.5×10^{-11} g Hg could be detected using only 1 μL of sample. The described method avoided many of the errors associated with sample pretreatment or preconcentration steps which were common in other analytical procedures. No reagents were added to samples, which prevented positive errors due to contamination. In addition, no analyte was lost due to volatilization during digestion procedures or due to incomplete extraction. Analysis was simple and rapid.

2. The carbon bed atomizer could be applied to the analysis of biological materials for other metals which are volatile at temperatures below 1550°C.

3. The average concentration of mercury in tap water from Baton Rouge was found to be 0.65 ppm. Tap water in the Chemistry Building at LSU contained 0.40 ppm Hg.

4. The average concentration of mercury in the urine of a sample population not occupationally exposed was 1.3 ± 0.9 ppm.

5. The average concentration of mercury in the sweat of a non-occupationally exposed population was 0.5 ± 0.4 ppm.

6. Mercury concentrations in urine and sweat were found to vary on a day-to-day basis. No correlation was found between mercury in urine and mercury in sweat for two individuals studied over an eight month period.

7. The average concentration of Hg in whole blood for a population not occupationally exposed was 0.43 ppm. The average concentration in serum was 0.32 ppm Hg for the same sample population.

8. Mercury in scalp hair seemed to arise from two sources: excretion from within the body and adsorption from environmental sources. Mercury found in the hair segments nearest the root most nearly represented excreted mercury, while that measured near the end of the hair strand reflected environmentally-contributed mercury as well. The mercury concentration increased with increased distance from the root.

9. The average concentration at the root of the hair was found to be 1.8 $\pm$ 1.5 ppm Hg for a group of non-occupationally exposed persons. This was considered to be a measure of the excretion of mercury from the body through the hair. The concentration of mercury at the root was significantly different between males and females.

10. The increase in mercury per unit length from distal end to proximal end of the hair strand differed between individuals. On the average, the increase per unit length for males was less than that for females. This indicated that mercury was being taken up by the hair from external sources.

11. The concentration of mercury in breath was found to have an average value of 2.65 $\mu g/m^3$ for a "normal" population.

12. The concentration of mercury in saliva was found to be 0.27 ppm in a "normal" population.

13. No correlation was observed between the concentrations of mercury in hair, urine, sweat, breath and saliva for a "normal" population. Good correlation was found between mercury concentrations in hair, breath and saliva for recent exposure.

14. The average daily excretion of mercury through body tissues and fluids could be estimated using the data accumulated in this research. These estimates were as follows:

urine: 2 mg Hg/day

sweat: 1.5 mg Hg/day

hair: 0.4 µg Hg/day

breath: 0.03 mg Hg/day

Therefore, the total daily excretion of mercury through these routes was about 3.53 mg/day.

There were other possible modes of excretion of mercury from the body; feces, nails, tears, skin exfoliation and mucous are a few examples. Feces are believed to be the major route of excretion of mercury which has passed through the gastrointestinal tract. The other modes of loss were considered to be minor compared to those of urine, feces and sweat.

The concentrations of mercury measured in these tissues in this study were higher than those reported in the literature. The estimated daily intake of mercury for a "normal" U.S. adult has been estimated to be 50 μg. It seemed that we were excreting more than we were taking in. However, the mercury levels measured in tap water in this study were also higher than those reported in the literature. It was thought to be a good assumption that much of the data on mercury concentrations in food and water were significantly low, due to analytical problems such as volatilization losses, incomplete extractions and failure to detect all of the forms of mercury in a sample.

15. The platinum loop and glass rod volatilization stages and quartz "T" atomizer exhibited promise as a means of speciation of mercury compounds in biological and environmental samples.

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VITA

Eileen Mary Skelly was born in Philadelphia, Pennsylvania on 15 January, 1953. She was graduated from Holy Child Academy, Sharon Hill, Pennsylvania in June, 1970. She studied chemistry at Drexel University in Philadelphia and received the Bachelor of Science degree with Highest Honors in June, 1975. Upon graduation, she was commissioned a Second Lieutenant in the United States Army Medical Service Corps and has served on active duty since that time. She was promoted to the rank of Captain in December, 1979 and was selected for civilian graduate training in chemistry. She began graduate studies in the Department of Chemistry, Louisiana State University, where she is currently a candidate for the degree of Doctor of Philosophy. Upon graduation, she will be assigned to the chemistry faculty at the United States Military Academy, West Point, New York.

EXAMINATION AND THESIS REPORT

Candidate: Eileen Mary Skelly

Major Field: Chemistry

Title of Thesis: The Direct Determination And Speciation Of Mercury Compounds In Environmental And Biological Samples By Carbon Bed Atomic Absorption Spectroscopy

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

October 18, 1982